Exhibit A

A sonographic screening method for Down syndrome

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We describe a potential screening method for the second trimester detection of the fetus with Down syndrome by the use of standard sonographic biometry. Retrospective assessment of the biparietal diameter, femur length, and biparietal diameter/femur length ratio in 55 fetuses with Down syndrome and 544 control fetuses was performed at two medical centers. Adequate numbers of cases and controls were available to permit statistically significant comparisons between the groups at 15 to 23 weeks' gestation. Fetuses with Down syndrome displayed no statistically significant differences in the cephalic index or the biparietal diameter compared with controls at a given gestational age. Significant femur length shortening was observed, but the greatest statistical difference was noted for the biparietal diameter/femur length ratio. This ratio was found to decrease with gestational age in the normal population and was consistently elevated in the Down syndrome population (compared with control) throughout the second trimester. With a cutoff value of 1.5 SD above the normal population mean for the biparietal diameter/femur length ratio, 50% to 70% of fetuses with Down syndrome could be identified in 6% of the total population ($p < 0.0001$). This preliminary study suggests that the biparietal diameter/femur length ratio may prove superior to current Down syndrome screening methods and, as a third independent variable, may significantly enhance the sensitivity and specificity of combined maternal age/maternal serum α-fetoprotein screening modalities. (Am J Obstet Gynecol 1987;157:803-8.)

Key words: Down syndrome, sonography, biparietal diameter, femur length, screening methods

The antenatal detection of Down syndrome before 24 weeks' gestation remains a major epidemiologic challenge. The sensitivity of maternal age–based Down syndrome screening programs has decreased as a result of a progressive decline in births to women over 34 years of age. Presently, <20% of Down syndrome cases are associated with a maternal age >34 years.[1] The introduction of screening techniques for Down syndrome that use decreased maternal serum α-fetoprotein concentrations enables assessment of the 95% of pregnant women <35 years old who are responsible for 80% of Down syndrome fetuses. However, such programs are also associated with a low sensitivity (13% to 21%) and high false-positive rate (7% to 9%).[2,3]

The increasing use of routine ultrasound in the second trimester has stimulated an interest in discovering a simple, readily reproducible sonographic method that identifies pregnancies at high risk for Down syndrome. Thus we performed a retrospective analysis of sonographically derived biometric differences between Down syndrome and normal fetuses at two prenatal diagnosis centers. A screening method exploiting the increased biparietal diameter/femur length ratio of Down syndrome fetuses is proposed.

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Material and methods

Control population. The ultrasound reports of all consecutive patients undergoing genetic amniocentesis at the Yale University Perinatal Unit who were between 15 and 23 weeks' gestation were reviewed for the period January 1, 1986, to October 1, 1986. Indications for amniocentesis included advanced maternal age (63%), abnormal maternal serum α-fetoprotein concentrations (34%), and a family history of genetic disorders (3%). Exclusion criteria included a discrepancy of >1.5 weeks between the menstrual age and gestational age derived from biparietal diameter and femur length measurements,[4,5] the failure to record both biparietal diameter and femur length measurements, or the presence of anatomic or chromosomal anomalies. A total of 349 patients was available for analysis in the New Haven control population.

Utilizing similar exclusion criteria, the ultrasound reports of 195 consecutive patients undergoing genetic amniocentesis for advanced maternal age and low ma-

Table I. Comparisons between cases and controls at 16, 18, and 20 weeks' gestation for the New Haven population

Variable	*Gestational age (wk)*	*Group*	*No.*	*Mean*	*SD*	*t test*	*Wilcoxon test*
Gestational age	16-17	DS	13	16.4	0.38		
		NL	172	16.4	0.35	$p < 0.64$	$p < 0.63$
	17.5-18.5	DS	10	18.1	0.36		
		NL	79	18.1	0.39	$p < 0.56$	$p < 0.59$
	20-21	DS	10	20.3	0.40		
		NL	26	20.3	0.28	$p < 0.85$	$p < 0.42$
Cephalic index	16-17	DS	5	0.82	0.03		
		NL	104	0.80	0.04	$p < 0.14$	$p < 0.09$
	17.5-18.5	DS	5	0.79	0.04		
		NL	57	0.80	0.04	$p < 0.85$	$p < 0.84$
	20-21	DS	6	0.82	0.05		
		NL	19	0.82	0.04	$p < 0.84$	$p < 0.75$
Biparietal diameter	16-17	DS	13	3.51	0.21		
		NL	172	3.60	0.22	$p < 0.13$	$p < 0.24$
	17.5-18.5	DS	10	4.13	0.32		
		NL	79	4.04	0.23	$p < 0.28$	$p < 0.13$
	20-21	DS	10	4.77	0.38		
		NL	26	4.73	0.30	$p < 0.72$	$p < 0.53$
Femur length	16-17	DS	13	1.87	0.24		
		NL	172	2.13	0.21	$p < 0.0001$	$p < 0.0002$
	17.5-18.5	DS	10	2.33	0.26		
		NL	79	2.59	0.21	$p < 0.0007$	$p < 0.006$
	20-21	DS	10	2.99	0.23		
		NL	26	3.29	0.28	$p < 0.003$	$p < 0.003$
Biparietal diameter/femur length ratio	16-17	DS	13	1.90	0.18		
		NL	172	1.71	0.14	$p < 0.0001$	$p < 0.0002$
	17.5-18.5	DS	10	1.77	0.20		
		NL	79	1.57	0.11	$p < 0.0001$	$p < 0.002$
	20-21	DS	10	1.61	0.06		
		NL	26	1.44	0.09	$p < 0.0001$	$p < 0.0001$

DS = Down syndrome; NL = normal.

ternal serum α-fetoprotein concentration at the Diagnostic Ultrasound Associates in Boston were reviewed for the period August 1, 1986 to October 1, 1986. Gestational ages ranged from 15 to 21 weeks.

Down syndrome population. All consecutive karyotypic diagnoses of trisomy 21 made at the Yale University Cytogenetics Laboratory from January 1, 1984, through October 1, 1986, were ascertained. Karyotype sources included amniotic fluid derived from genetic amniocenteses (91%) and lymphocytes from neonates with Down syndrome stigmatas (9%). Those women bearing Down syndrome fetuses who had undergone second-trimester obstetric ultrasound examinations at the Yale Perinatal Unit before the diagnosis of Down syndrome were identified. Exclusion criteria consisted of intrauterine fetal death, hydrocephalus, inaccurate dating criteria, or a failure to record both biparietal diameter and femur length measurements. Ultrasound data were available in 35 patients from 15 to 23 weeks' gestation.

Consecutive karyotype diagnoses of trisomy 21 made after genetic amniocenteses at the Diagnostic Ultrasound Associates were identified for the period January 1, 1985, through October 1, 1986. Exclusion criteria were similar. Data were available from 20 patients with Down syndrome fetuses from 15 to 19 weeks' gestation.

Ultrasound techniques and equipment. The biparietal diameter was measured with electronic calipers from leading edge to leading edge at the level of the thalami. The femur was preferentially visualized from the greater trochanter to the end of the ossified shaft, perpendicular to the long axis of the bone, and its length was recorded with electronic calipers. The cephalic index was calculated by dividing the biparietal diameter by the occipitofrontal diameter measured at the same level.

The ultrasound equipment used in New Haven included the General Electric models RT 2600 and 3000 (3.5 and 5.0 mHz linear array transducers; Rancho Cardova, California), the Advanced Technology Laboratories Ultramark 4 (3.5 and 5.0 mHz linear array transducers; Botnell, Washington), and the Acuson 128 (3.0 mHz linear array transducer). The equipment used in Boston included an Acuson 128 or ATL 300 (3.0 and 3.5 mHz sector transducers). To ensure an accurate estimate of the femur length measurements with the sector scanner, the femur lengths were repeatedly imaged and the longest possible length was recorded.

Data collection and statistical analysis. Ultrasoun

Table II. Comparisons between cases and controls at 16 and 17 weeks' gestation for the Boston population

Variable	*Gestational age (wk)*	*Group*	*No.*	*Mean*	*SD*	*t test*	*Wilcoxon test*
Gestational age	15.0-16.5	DS	8	15.7	0.47		
		NL	71	15.7	0.34	$p < 0.73$	$p < 0.95$
	16.6-17.5	DS	8	16.9	0.42		
		NL	80	16.8	0.37	$p < 0.50$	$p < 0.52$
Biparietal diameter	15.0-16.5	DS	8	3.31	0.12		
		NL	71	3.31	0.12	$p < 0.98$	$p < 0.86$
	16.6-17.5	DS	8	3.65	0.12		
		NL	80	3.62	0.13	$p < 0.57$	$p < 0.62$
Femur length	15.0-16.5	DS	8	1.85	0.19		
		NL	71	2.10	0.15	$p < 0.0001$	$p < 0.001$
	16.6-17.5	DS	8	2.15	0.24		
		NL	80	2.38	0.14	$p < 0.0001$	$p < 0.009$
Biparietal diameter/femur length ratio	15.0-16.5	DS	8	1.80	0.12		
		NL	71	1.59	0.10	$p < 0.0001$	$p < 0.0002$
	16.6-17.5	DS	8	1.71	0.17		
		NL	80	1.52	0.08	$p < 0.0001$	$p < 0.002$

DS = Down syndrome; NL = normal.

reports were reviewed and the indications for evaluation, gestational age, biparietal diameter, femur length, and cephalic index were recorded. The calculation of the cephalic index was not available for the Boston population. Hadlock's formula[6] and Jeanty's formula[7] were used in the estimation of gestational age based on biparietal diameter and femur length, respectively.

The biparietal diameter, femur length, biparietal diameter/femur length ratio, and cephalic index were compared in the Down syndrome and control populations at given gestational ages. Differences between cases and controls were identified by Student's *t* test and a Wilcoxon signed rank test. The ability of specific biparietal diameter/femur length cutoff values to discriminate between Down syndrome and normal fetuses was assessed by χ^2 analysis and Fisher's exact test. The effect of gestational age on the biparietal diameter/femur length ratio was assessed by general linear model regression analysis.

Results

There was a consistent underestimation of the femur length for a given gestational age in the New Haven population, and a consistent overestimation in the Boston population when compared with Jeanty's nomogram.[7] The differences between the two centers were statistically significant ($p < 0.0001$) from 16 to 19 weeks' gestation, and precluded combining the two centers' data sets.

New Haven results. Power analysis[4] demonstrated that adequate numbers of cases and controls were available to allow for statistically meaningful (>80%) comparisons from 16 to 17, 17.5 to 18.5, and 20 to 21 weeks' gestation (Table I). Other gestational week intervals contained insufficient cases. No statistically significant differences were detected between cases and controls for mean gestational age, suggesting similar gestational age distributions within the chosen intervals. No statistically significant differences were noted for cephalic index or biparietal diameter. There was, however, a statistically significant shortening of femur lengths in the Down syndrome fetuses in each gestational week interval. Furthermore, the biparietal diameter/femur length ratio resulted in the highest statistically significant differences between the two populations. A Wilcoxon signed rank test confirmed the statistically significant differences for femur length and particularly for the biparietal diameter/femur length ratio (Table I).

Boston results. Identical findings were noted for the Boston center (Table II). Gestational age intervals were again chosen to maximize power (>80%) and included 15.0 to 16.0 and 16.5 to 17.5 weeks' gestation. No significant differences were noted for gestational age or biparietal diameter between the case and control groups. Statistically significant differences were noted for femur length and particularly the biparietal diameter/femur length ratio between the two groups.

Variation in biparietal diameter/femur length ratios across gestational age. The biparietal diameter/femur length ratio (BPD/DL) was noted to decrease across gestational age (GA) in both centers' control populations according to the following formulas:

$$BPD/FL = 3.222 - 0.125\ (GA) + 0.002\ (GA)^2$$
$$BPD/FL = 2.406 - 0.052\ (GA)$$
$$(R^2 = 0.56;\ p < 0.0001;\ \text{New Haven})$$
$$(R^2 = 0.36;\ p < 0.0001;\ \text{Boston})$$

The biparietal diameter/femur length ratio was consistently higher in the Down syndrome population at

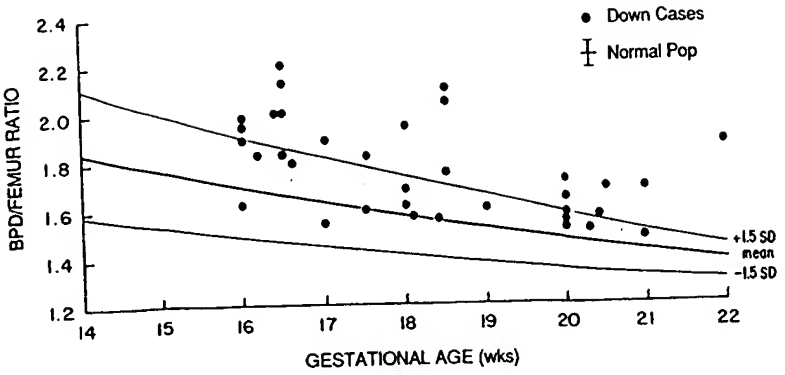


Fig. 1. The biparietal diameter/femur length ratio *(BPD/FEMUR)* in the normal population (±1.5 SD) and in Down syndrome cases for the New Haven population.

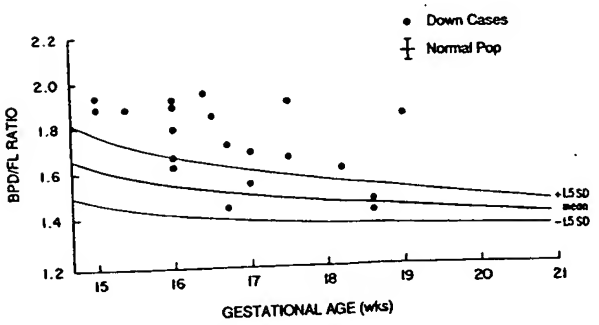


Fig. 2. The biparietal diameter/femur length ratio *(BPD/FL)* in the normal population (±1.5 SD) and in Down syndrome cases for the Boston population.

all gestational ages. For the New Haven population, 51% of Down syndrome cases were found to have a biparietal diameter/femur length ratio 1.5 SD above the control population mean for a given gestational age, while only 6% of Down syndrome fetuses were below the mean (Fig. 1). For the Boston population, 70% of Down syndrome fetuses were 1.5 SD above the control population mean, while only 10% were at or below the mean (Fig. 2).

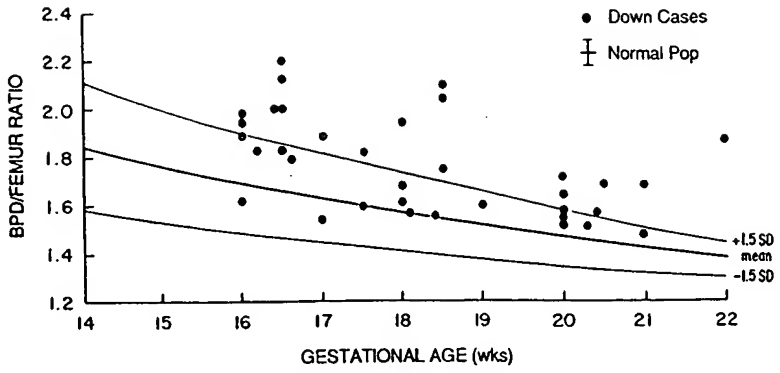
Predictive value of biparietal diameter/femur length ratio in discriminating between Down syndrome and normal fetuses. The sensitivity and false positive rate of the biparietal diameter/femur length ratio in differentiating Down syndrome from normal fetuses were also evaluated for each center (Table III). With a cutoff value of 1.5 SD above the mean ratio for the normal New Haven population, 51% of Down syndrome fetuses were identified with a false positive rate of 7%. Given a general population incidence for Down syndrome at term of 1/1000[6] and a fetal loss rate of 29%,[5] the incidence of Down syndrome in the second trimester should be 1/710 and the resulting positive predictive value of the proposed biparietal diameter/femur length ratio cutoff would be 1/103. Thus 102 amniocenteses on non–Down syndrome gestations would be necessary for each Down syndrome diagnosis. In a high-incidence population, such as women 35 years old, the anticipated incidence of Down syndrome at the time of genetic amniocentesis would be 1/270,[8] and thus 37 amniocenteses would be required for one diagnosis of Down syndrome (positive predictive value 1/37).

For the Boston center, a cutoff value of 1.5 SD above the mean ratio for the control population would identify 70% of cases with a false-positive rate of 4.6%. The positive predictive value for the general obstetric population would be 1/47 and that for women at age 35 years would be 1/18.

The ability of the femur length measurement alone to differentiate Down syndrome from normal fetuses was also assessed. With a cutoff value of 1.5 SD below the control (New Haven) population mean, 40% of Down syndrome fetuses were identified, while for the Boston center 60% of Down syndrome fetuses were identified. Femur length, therefore, proved less accurate than the biparietal diameter/femur length ratio in discriminating Down syndrome fetuses.

Comment

Recently, characteristic sonographic "signs" for the Down syndrome fetus have been proposed as adjuncts to screening programs. The sonographic finding of posterior occipital-nuchal skin thickening may be present in up to 45% of Down syndrome fetuses in the second trimester.[9] The practical application of this observation remains to be determined. In addition, duodenal obstruction occurs in 8% of Down syndrome infants[10] and has been proposed as a marker for the condition.[11] Unfortunately, the literature suggests that the diagnosis of duodenal obstruction can rarely be made before 24 weeks' gestation.[12] Ventricular septal and atrioventricular canal defects are present in up to 50% of Down syndrome infants,[13] but it is not clear



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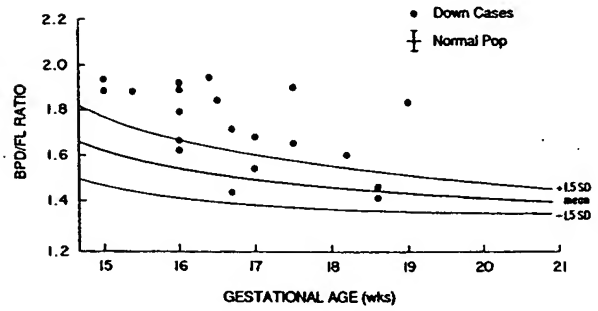

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Table III. Sensitivity and false-positive rate of the biparietal diameter/femur length ratio in the detection of Down syndrome

Test center	*Gestational age (wk)*	*No.*	*Cutoff value (mean + 1.5 SD)*	*Overall sensitivity*	*Overall false positive rate*	*p value**
New Haven	15.0-15.9	27	1.93			
	16.0-16.9	141	1.93			
	17.0-17.9	64	1.76			
	18.0-18.9	53	1.74	18/35	26/349	<0.0001
	19.0-19.9	27	1.68	(51%)	(7%)	<0.0001
	20.0-20.9	25	1.58			
	21.0-21.9	8	1.54			
	22.0-22.9	4	1.47			
Boston	15.0-15.9	36	1.77			
	16.0-16.9	78	1.66			
	17.0-17.9	50	1.61	14/20	9/195	<0.0001
	18.0-18.9	16	1.54	(70%)	(5%)	<0.0001
	19.0-19.9	11	1.51			
	20.0-20.9	4	1.48			

*χ^2 or Fisher's exact test.

what percentage of these defects can be identified with an ultrasound screening examination. The use of standard ultrasound biometry to differentiate Down syndrome from normal fetuses has great appeal. Both biparietal diameter and femur length are readily and accurately measured during a basic ultrasound examination. These measurements require neither sophisticated training nor expensive ultrasound equipment.

Initial efforts to define a biometric marker of Down syndrome used the long-standing observation that Down syndrome infants tend toward brachycephaly (increased cephalic index).[14] Perry et al.,[15] however, were not able to differentiate normal from Down syndrome fetuses on the basis of an increased cephalic index during second-trimester sonography. Our data confirm their observation, with no difference in cephalic index noted between case and control populations (Table I). In addition, we have observed that while femur lengths were significantly shorter in Down syndrome fetuses, consistent with their shorter stature,[16] femur length per se was not the optimal discriminator. By combining biparietal diameter and femur length measurements into a ratio, discrimination between Down syndrome and normal fetuses is enhanced. This finding is in agreement with the observations of Collett et al.,[17] who noted that the biparietal diameter/femur length ratio was elevated in fetal Down syndrome. This suggests that in the normal population, shorter femur lengths are associated with smaller biparietal diameters, perhaps as a consequence of inaccurate dating or symmetric growth retardation. Because the biparietal diameter/femur length ratio is far less affected by changes in gestational age than is femur length alone, it should provide a more reliable clinical marker for Down syndrome.

The use of the biparietal diameter/femur length ratio with a 1.5 SD cutoff, between 15 and 23 weeks' gestation, allows for the detection of Down syndrome in the general obstetric population with a sensitivity of 50% to 70%, greater than that of either low maternal serum α-fetoprotein concentration (≤0.5 multiple of the median) or maternal age–based (>34 years) screening programs alone (<20% each). In addition, the positive predictive value exceeds that of maternal age–based screening; thus a far larger number of Down syndrome fetuses are identified with fewer unnecessary amniocenteses.

A number of potential limitations are present in this study. The Down syndrome sample size is small. However, a power analysis suggests >90% probability of identifying a true difference between the Down syndrome and control populations.[18] An additional source of error may result from the tendency for sonographers to minimize gestational age discrepancies between biparietal diameter and femur length measurements. This phenomenon could cause a significant reduction in the ratio's sensitivity for the detection of Down syndrome, because the greater the biparietal diameter/femur length discrepancy, the greater the likelihood of remeasurement.

The difference in femur length measurements between the two centers probably reflects differences in methodology and not variations in population. The use of a sector transducer results in a relative overestimation of long bone length, in a plane perpendicular to the bone's long axis, when compared with the linear array transducer.[19] Furthermore, while the greater trochanter provides a ready landmark for measurement, sonographers may vary in their assessment of the distal termination of the femoral diaphysis. We postulate that an overestimation of femur length accounts for the Boston center's greater diagnostic sensitivity. The smaller biparietal diameter/femur length ratio in the Boston control population may paradoxically amplify

the effect of femur length shortening in the Down syndrome population. It is therefore crucial that each center establish its own biparietal diameter/femur length limits of confidence in the second trimester for Down syndrome risk assessment.

The role of the biparietal diameter/femur length ratio in prenatal screening for Down syndrome remains to be confirmed by further investigations. Variation in ultrasound equipment and technique may lead to significant effects on a given center's diagnostic efficacy. Nonetheless, this method appears at least comparable to methods that use maternal serum α-fetoprotein and/or maternal age. Since Baumgarten et al.[20] have demonstrated that ascertainment of Down syndrome risk by a combination of maternal serum α-fetoprotein level and maternal age may improve diagnostic accuracy, the addition of a third independent predictor (biparietal diameter/femur length ratio) of Down syndrome risk could further enhance this diagnostic accuracy. The effect of the biparietal diameter/femur length ratio on the relative risk of Down syndrome could be assessed and used to provide a patient with an adjusted risk based on all three parameters.

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Exhibit B

ELSEVIER

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Early Human Development

Brachycephaly is ineffective for detection of Down syndrome in early midtrimester fetuses

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Abstract

The aim of this study was to assess the value of fetal brachycephaly in the detection of Down syndrome at 13–18 weeks of pregnancy. The cephalic index (CI) was determined in 555 consecutive chromosomally normal fetuses, and in 38 chromosomal abnormalities, prior to amniocentesis. A CI > 0.85 was observed in 14% (2/14) of the fetuses with Down syndrome and in 11% of the normal fetuses. In conclusion, our data show that brachycephaly is not a useful marker for Down syndrome in early midtrimester fetuses.

Keywords: Brachycephaly; Down syndrome screening; Ultrasonography; Prenatal diagnosis

1. Introduction

One of the neonatal features of Down syndrome is brachycephaly, which has been found to be present in most of the affected infants [1]. Prenatally, brachycephaly has been suggested as an ultrasound marker, when the only case with a marked brachycephaly among 53 midtrimester fetuses was found to have Down syndrome

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[2]. Subsequent studies have assessed the value of fetal brachycephaly in the prenatal detection of Down syndrome, with controversial results [3–6].

In order to establish the role of fetal brachycephaly in the detection of Down syndrome, we have studied 555 consecutive pregnant women undergoing amniocentesis, subsequently showing chromosomally normal fetuses, and 38 pregnancies with chromosomal abnormalities, 14 of which were Down syndrome

2. Material and methods

Five-hundred fifty-five consecutive early midtrimester fetuses were studied prior to amniocentesis, in our Prenatal Diagnosis Unit, between January and October 1994. The indications for amniocentesis were as follows: (a) positive biochemical screening ($n = 212$), (b) advanced maternal age ($n = 203$), (c) anxiety ($n = 68$), (d) risk for neural tube defect ($n = 37$), (e) fetal malformation ($n = 27$), (f) previous chromosomal abnormality ($n = 6$), (g) DNA studies ($n = 1$), and (h) parental chromosomal abnormality ($n = 1$).

Maternal age ranged from 18 to 42 years, (mean 35.2 and 95% confidence intervals: 34.9–35.5). Gestational age ranged from 13 to 18 weeks (mean 15.6 and 95% confidence intervals: 15.5–16.0).

Ultrasound examination was performed without knowledge of the fetal karyotype (Hitachi EUB 515A, Hitachi Medical Corporation, Tokyo, Japan). The biparietal (BPD) and the occipito-frontal diameter (OFD) were measured in a standard fashion. The cephalic index (CI) was calculated as the ratio BPD/OFD. Brachycephaly was defined either by a $CI > 0.85$, or a $CI >$ 95th percentile.

Thirty-eight consecutive cases of chromosomal abnormalities were detected in our series of 1460 amniocenteses, performed between January 1994 and December 1995, 14 of which were Down syndrome. The CI was also determined prior to the procedure, without knowledge of the fetal karyotype. We established the sensitivity of fetal brachycephaly to detect Down syndrome and the false positive rate (1 − specificity). The positive predictive value (PPV) was adjusted to the prevalence of Down syndrome in the general population (EUROCAT) [7] following the Bayes' theorem.

3. Results

In the chromosomally normal population the mean CI was 0.79 (95% confidence intervals: 0.79–0.80), ranging from 0.64 to 0.97 (Table 1). Seventy-four percent of the cases showed a normal CI, defined as between 0.75 and 0.85, and 11% were brachycephalic ($IC > 0.85$). The 5th and 95th percentiles were 0.71 and 0.89, respectively.

Mean CI in the 14 cases of Down syndrome was 0.81 (95% confidence intervals: 078–0.84), ranging from 0.72 to 0.89 (Table 1). Only two (14.3%) of the cases had a $CI > 0.85$, but none was over the 95th percentile. Thus, brachycephaly showed a

Table 1
Mean cephalic index (CI) and number of cases selected either by CI > 0.85 or CI > 95th percentile, in each group of fetal karyotype

Fetal karyotype	*n*	CI mean (95% confidence intervals)	CI > 0.85	CI > 95th percentile
Normal	555	0.79 (0.79–0.80)	61 (11)	28 (5)
Trisomy 21	14	0.81 (0.78–0.84)	2 (14)	0
Trisomy 18	4	0.79 (0.70–0.88)	0	0
Sexual trisomies	4	0.76 (0.71–0.81)	0	0
Marker chromosome	1	0.83	0	0
Triploidy	4	0.84 (0.74–0.95)	2 (50)	2 (50)
Mosaicisms	2	0.80 (0.52–1.08)	0	0
Balanced rearrangements	7	0.79 (0.76–0.82)	0	0
Unbalanced rearrangements	2	0.80 (−0.3–1.64)	1 (50)	0

sensitivity of 14.3% in the prediction of Down syndrome, for an 11% false-positive rate. The PPV was 0.2% (1:526) when adjusted to the prevalence of Down syndrome in the general population. When using the 95th percentile instead of a IC > 0.85 to define fetal brachycephaly, sensitivity was nil.

In relation to other chromosomal abnormalities, only two out of four triploidies and one out of two unbalanced rearrangements showed a CI > 0.85 (Table 1). When considering the 95th percentile, only the two triploidies were detected.

4. Discussion

Brachycephaly is characterized by a marked flattening of the occiput and a relative shortening of the OFD. It is found in association with chromosomal and monogenic defects. In infants born with Down syndrome brachycephaly is recognized as one of the main features.

Fetal brachycephaly was suggested as a prenatal ultrasound marker for Down syndrome by Buttery (1979) [2], when the one single case showing brachycephaly among 53 midtrimester fetuses, was subsequently found to have Down syndrome. Since determination of the CI (BPD/OFD) needs only the additional ultrasound measurement of the OFD, it might provide a suitable marker for screening purposes.

Three further studies have not supported the value of fetal brachycephaly as an ultrasound marker in the second trimester of pregnancy. In a prospective study, Perry et al. (1984) [3] found that the CI in eight fetuses with Down syndrome was indistinguishable from that in 308 chromosomally normal fetuses (0.825 vs. 0.829). Similarly, Lockwood et al. (1987) [4] did not find differences in the retrospective comparison of 18 cases of Down syndrome with 180 control fetuses (0.81 vs. 0.80). In another retrospective study, Shah et al. (1990) [5] found no significant difference in CI in 17 cases of Down syndrome when compared to 17 matched controls (0.80 vs. 0.78).

In our series, brachycephaly does not appear to be a useful marker, since it is present in 14% of Down syndrome fetuses and in 11% of the controls. The

comparison between both groups did not show a significative difference in CI (0.81 vs. 079) (Table 1). In relation to other chromosomal abnormalities, the triploid fetuses were the group in which brachycephaly was more commonly present (50%).

Brachycephaly, defined as a CI > 97.5th percentile, was observed by Snijders and Nicolaides (1995) [6] in a reduced number of fetuses with triploidy (10%) and Down syndrome (15%), but in a slightly higher percentage in those with trisomies 13 and 18 (28%), and in those with Turner syndrome (32%). It may be argued that the improved value of fetal brachycephaly in this study to detect Down syndrome may be due to the wider gestational age studied, which includes both the second and the third trimester (16–40 weeks). Although a gestational age effect may be suspected since the presence of brachycephaly is more common at term than in midtrimester fetuses, no such impact has yet been reported by ultrasound studies [8].

Since the described shortening of OFD is the result of reduced frontal lobe growth, Bahado-Singh et al. (1992) [9] suggested the ultrasound measurement of the fronto-thalamic distance to improve the prenatal detection of Down syndrome. In this retrospective study, 21% of the fetuses with Down syndrome and 4.8% of the normal cases were selected, at 16–21 weeks.

A complementary ultrasound scan is recommended to improve the results of the biochemical screening, using two different approaches. Firstly, after a positive screen, to reassess gestational age and additionally to rule out any other cause of altered biochemical levels [10]. Secondly, for routine pregnancy dating prior to maternal sampling [11]. In both situations the evaluation of ultrasound markers may be useful in order to increase either the specificity or sensitivity, respectively, for Down syndrome. However, our data show that fetal brachycephaly is not a useful marker for Down syndrome at 13–18 weeks of pregnancy.

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Exhibit C

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Early Transvaginal Measurement of Cephalic Index for the Detection of Down Syndrome Fetuses

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Key Words
Brachycephaly · Down syndrome · Transvaginal sonography · Prenatal diagnosis

Abstract
Objective: The aim of this study was to produce normal cephalic index values by transvaginal scan early in pregnancy and to evaluate the screening utility of this measurement for the identification of fetuses at risk for Down syndrome. ***Method:*** In this prospective cross-sectional study, transvaginal high-resolution sonography was performed between 9 and 16 weeks of gestation in 1,087 euploid fetuses and 36 Down syndrome fetuses. Measurements of the cephalic index, calculated as the ratio biparietal diameter/occipito-frontal diameter, were plotted against gestational age. ***Results:*** The cephalic index was found to show fairly constant values throughout the period evaluated. The best correlation with gestational age was described by a linear correlation. All the measurements obtained in Down syndrome fetuses were within the normal ranges. ***Conclusion:*** Our data show that in early pregnancy the cephalic index cannot be considered a useful tool in the detection of fetuses at risk for Down syndrome.

Introduction

Most fetuses with major chromosomal abnormalities have either external or internal defects which can be recognized by detailed transabdominal scan in the second trimester [1–3]. In cases of trisomies 13 and 18, Turner's syndrome and triploidy, what we would consider as ultrasound markers are often major and multiple defects [4, 5]. In contrast, in Down syndrome (DS) fetuses the structural defects are subtle and often isolated [6 ,7]. Considering that most trisomic 21 infants recently investigated by transabdominal scan were found to have a brachycephalic head [8, 9], the validity of this biometric parameter in the prenatal detection of DS has been investigated.

Since transvaginal ultrasound provides an important and powerful tool with which to investigate early pregnancy, allowing a better and earlier visualization of embryo-fetal organs and structures than the transabdominal route [10, 11], we investigated and established the role and screening utility of the cephalic index (CI) in early pregnancy.

Patients and Methods

We retrospectively studied 36 DS fetuses (study group) and 1,087 euploid fetuses (control group) at 9–16 weeks of gestation referred to our Prenatal Unit for prenatal diagnosis between April 1994 and March 1998. Maternal age was over 37 years. Gestational age (GA)

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was estimated on the basis of the first day of the last menses as referred by the patient, a history of regular cycles lasting from 24–32 days and an early positive pregnancy test.

All scans were performed by the authors (P.R. and L.G.) using a high-resolution apparatus (Toshiba SSA-270 A and SSA-340 A, Toshiba Medical Corporation, Tokyo, Japan) equipped with 5.0 and 6.0 MHz probes with a maximal angle of vision of 86 and 121°, respectively.

In all cases a karyotype analysis was obtained by transabdominal amniocentesis performed between 15 and 18 weeks of gestation, and the results were unknown to the sonographers at the time of the scan.

The biparietal diameter (BPD) and the occipito-frontal diameter (OFD) were measured in the standard fashion and, in particular, the OFD was measured in the transverse head plane from the outer-to-outer edges of the occipital and frontal bones. The CI was calculated as the ratio BPD/OFD. Brachycephaly was defined by a CI >97.5 percentile, in agreement with Snijders and Nicholaides [2]. The CI for our normal controls was calculated and compared with the data obtained in DS fetuses.

Statistical analyses included Student's t test. $p < 0.01$ was considered significant for a two-tailed distribution.

Results

CI values in euploid fetuses were relatively consistent throughout the period of pregnancy investigated and the best model for assessing the relationship between the CI and the GA was found to be one derived from a linear regression equation ($CI = 0.005, GA + 0.76; r^2 = 0.007$). A nomogram with confidence intervals (2.5th and 97.5th percentile) established for CI versus GA in the control fetuses was graphed and the individual values for DS fetuses plotted (fig. 1). It can be seen that the CI values of DS fetuses were in the normal ranges in early pregnancy and none of the trisomic 21 fetuses were above the cutoff value of the 97.5th percentile.

The mean CI was 0.82 (97.5% confidence interval 0.72–0.92) in the population with normal fetal karyotypes and 0.82 (97.5% confidence interval 0.77–0.87) in the 36 cases of DS fetuses (p = n.s.).

Discussion

Nowadays, the objectives of both transvaginal and transabdominal scans include also the evaluation of abnormalities or biometric parameters as markers for the identification of fetuses at risk for chromosomopathies [2, 6, 12, 13]. Brachycephaly, implying that the head is relatively wide from side to side (BPD) and narrow from front to back (fronto-occipital diameter), is a characteristic feature of DS [14] and this feature has been found in most

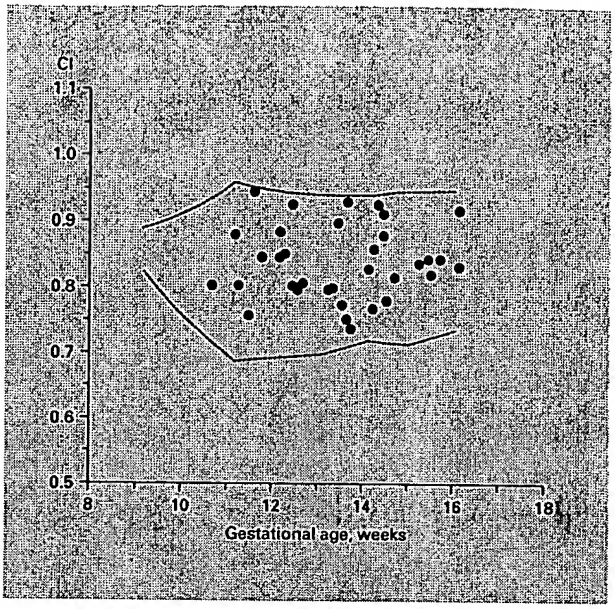

Fig. 1. Ultrasonographically based cephalic index (CI) versus gestational age (2.5th and 97.5th percentile). ● = Fetuses with trisomy 21.

affected infants [8]. Prenatally, trisomy 21 is associated with a tendency towards brachycephaly [2] but the results obtained by ultrasound examination are still controversial [2, 9, 15]. Among the cephalic measurements obtained in fetuses with abnormal karyotype, Seoud et al. [4] found that trisomic fetuses in general had smaller BPD and head circumference. Perry et al. [16] and Lockwood et al. [17] reported no statistically significant difference between trisomic and control fetuses in the incidence of brachycephaly. Snijders and Nicholaides [2] observed brachycephaly in a reduced number of fetuses with triploidy and DS but in a slightly higher percentage in those with trisomies 13 and 18 and those with Turner syndrome.

In our previous report [18] we found no statistically significant differences in cephalic biometry, considered as a ratio, between chromosomopathic fetuses and controls. Only in trisomic 13 fetuses were values abnormal, probably due to the high incidence of microcephaly.

To our knowledge there are no data in the literature regarding biometric evaluation of CI in early pregnancy that may be considered to constitute a pattern, even if controversially associated with DS. Our data, obtained transvaginally in the early stages of pregnancy, indicate that brachycephaly cannot be considered a useful tool in the detection of fetuses at risk for DS.

It is possible that the results concerning the effectiveness of early transvaginal ultrasound in our study may be biassed by the small number of DS cases. Moreover, biologic differences between trisomy 21 fetuses and unaffected fetuses may not be of sufficient magnitude to permit discrimination by ultrasonography in the first and early second trimester of pregnancy and the noxa may affect the cephalic structures later in pregnancy. Additional studies are required to further evaluate the validity of CI in early pregnancy as a biometric marker of aneuploidy.

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Exhibit P

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TRANSACTIONS OF THE ELEVENTH ANNUAL MEETING OF THE SOCIETY OF PERINATAL OBSTETRICIANS

(Contents continued on page 4A)

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Comparison of humerus length with femur length in fetuses with Down syndrome

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A recent report by FitzSimmons et al. demonstrated a greater frequency of upper- versus lower-extremity shortening in autopsies of second-trimester fetuses with trisomy 21. We undertook this study to determine whether this upper-limb shortening could be detected by prenatal ultrasonography in the second trimester and therefore identify fetuses at risk for trisomy 21. A restospective review of all prenatal sonograms preceding genetic amniocentesis was conducted. Between 1987 and 1990 11 consecutive fetuses between 15 and 22 weeks' gestation with trisomy 21 were identified by genetic amniocentesis. Femur and humerus lengths were plotted on growth curves created from 1470 normal patients between 12 and 26 weeks. Gestational age was confirmed by last menstrual period and biparietal diameter. In fetuses with trisomy 21, seven of 11 humeri were <5th percentile, for a sensitivity of 64%, whereas only two of 11 femurs were <5th percentile, for a sensitivity of 18%. Biparietal diameter/femur length and biparietal diameter/humerus length ratios were also tested to predict Down syndrome. In only 2 of 11 cases was the biparietal diameter/femur length ratio >95th percentile, whereas the biparietal diameter/humerus length ratio was >95th percentile in 7 of 11. Since all seven were identified by shortened humerus alone, we conclude that humerus length versus gestational age is the simplest and most effective screen. The positive predictive value of an abnormally short humerus length in detecting Down syndrome was 6.8% in our population where the prevalence of Down syndrome was 1 of 173. The present study supports the observations of FitzSimmons et al. that shortened humerus length has a greater sensitivity than femur length in cases of trisomy 21. We conclude that in fetuses at risk for trisomy 21 humerus length should be determined, because it may, if shortened, aid in the prenatal diagnosis. (AM J OBSTET GYNECOL 1991;165:1051-6.)

Key words: Down syndrome, prenatal diagnosis, humerus length

The prenatal diagnosis of Down syndrome has relied primarily on genetic amniocentesis or, more recently, on chorionic villus sampling, in women ≥35 years of age. Even if all women 35 or older underwent prenatal diagnostic testing, approximately 80% of Down syndrome would not be detected, because it occurs in the 95% of childbearing women that are under 35. Moreover, many women (even those at high risk) choose not to have invasive genetic testing for fear of miscarriage following the procedure. Therefore a noninvasive test to either detect or rule out Down syndrome would be beneficial. Ultrasonographically detected abnormalities associated with Down syndrome include increased nuchal skin thickening,[1] short femur length, abnormal biparietal diameter/femur length ratio,[2] duodenal atresia,[3] omphalocele,[4] cardiac defects,[5] cystic hygromas,[6] and nonimmune hydrops.[7] However, these findings are not consistently found in most fetuses with Down syndrome. FitzSimmons et al.,[8] using radiographs of embryo specimens, recently described long-bone growth in cases of Down syndrome and reported that shortening of the upper extremity was more pronounced than that of the lower extremity. We undertook this study to determine if the obstructions of shortened humeri of FitzSimmons et al.[8] could be extended to ultrasonographic observations and thus to determine if fetal humerus length measurements in the second trimester could be helpful in detecting Down syndrome prenatally. Therefore the purpose of this study was to make second-trimester nomograms for humerus length and biparietal diameter/humerus length ratio versus gestational age and humerus length versus biparietal

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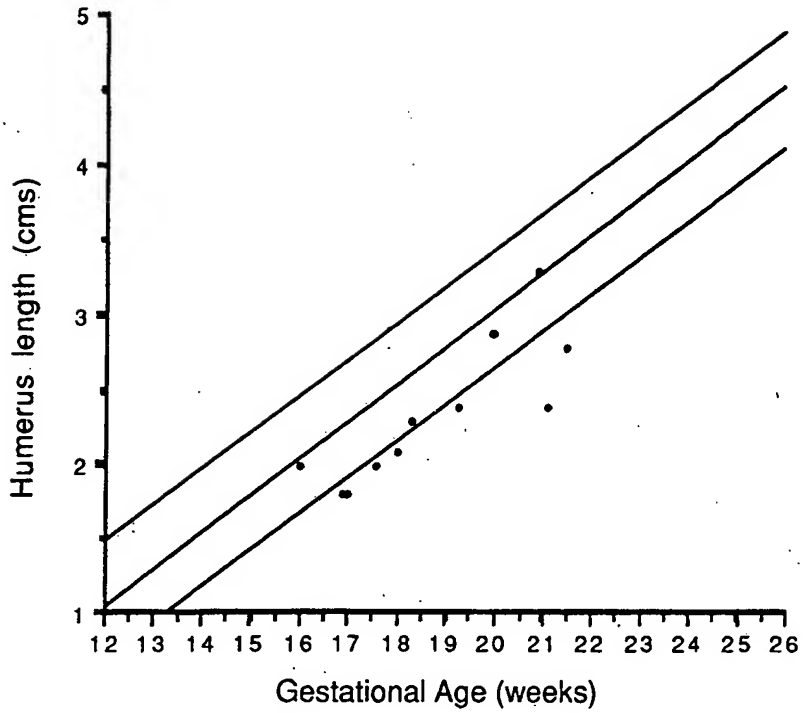

Fig. 1. Relationship between humerus length and gestational age in second trimester. Shown are 5th, 50th, and 95th percentiles. *Closed circles,* Fetuses with Down syndrome.

diameter in our population, to retrospectively identify fetuses with Down syndrome detected by genetic amniocentesis, to determine the sensitivity of humerus length and femur length to identify Down syndrome at different cutoff values (e.g., 5th and 10th percentiles), and to determine efficacy of an abnormal humerus length to predict Down syndrome in our population of genetic amniocentesis over a 2½-year period.

Material and methods

Nomograms were established from 1470 ultrasonographic examinations performed between 12 and 26 weeks' gestation in patients with a certain last menstrual period. In all cases the estimated gestational age, as established by last menstrual period, was within 1.5 weeks of the gestational age estimated by ultrasonography. Measurements obtained at each examination included biparietal diameter, head circumference, femur length, humerus length, and abdominal circumference. Fetuses with known congenital or chromosomal anomalies were excluded. This portion of the study was cross-sectional; patients were included once. All examinations were performed between Jan. 1, 1988, and June 30, 1990, by members of the Division of Maternal-Fetal Medicine at the University of Connecticut Health Center and Hartford Hospital with General Electric RT 3000 (Milwaukee) and Acuson 128 (Mountain View, Calif.) real-time ultrasonography machines with freeze-frame capabilities and on-screen calipers. Linear and polynomial regression analyses were performed to describe the relationships between humerus length versus gestational age, humerus length versus biparietal diameter, biparietal diameter/humerus length ratio versus gestational age, and biparietal diameter/femur length ratio versus gestational age. The 5th, 10th, 50th, 90th, and 95th percentiles were calculated. All patients who come through our antepartum testing unit are given outcome cards to complete; about 75% return these.

Records of all patients undergoing second-trimester genetic amniocenteses over the 2½-year study period were reviewed to identify all cases of Down syndrome diagnosed prenatally and to determine the prevalence of Down syndrome in this population. Over this study period, 1907 genetic amniocenteses were performed. Indications included advanced maternal age (77%), low maternal serum α-fetoprotein value (20%), and previous child with trisomy (3%). At the time of amniocentesis, ultrasonographic measurements including biparietal diameter, head circumference, femur length, humerus length, and abdominal circumference were obtained.

Results

During the study period 11 fetuses with Down syndrome were identified by second trimester genetic am-

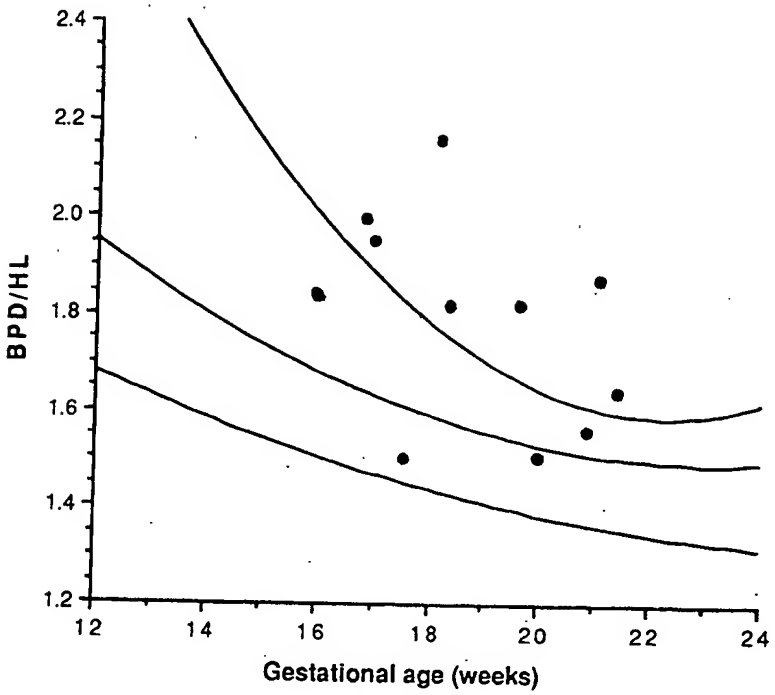

Fig. 2. Relationship between biparietal diameter/humerus length *(BPD/HL)* ratio versus gestational age. Shown are 5th, 50th, and 95th percentiles. *Closed circles,* Fetuses with Down syndrome.

niocentesis. We compared the humerus length in these 11 cases with the curves established in the 1470 normal patients (Fig. 1) and found that in seven of 11 cases (sensitivity, 64%) the humerus length fell below the 5th percentile from the norm for that gestational age. If the 10th percentile was chosen as a cutoff, the sensitivity remained at 64%. In all 11 cases, the humerus length fell below the 50th percentile. The relationship between humerus length and gestational age is described by the linear equation humerus length = 0.241 gestational age − 1.787 (r = 0.939, R^2 = 0.88, p = 0.0001). The biparietal diameter/humerus length ratio versus gestational age is illustrated in Fig. 2; the relationship is described by the second-order polynomial equation biparietal diameter/humerus length = 3.834 − 0.204 gestational age + 0.004 gestational age^2 (r = 0.517, R^2 = 0.26, p = 0.0001). Seven of 11 fetuses with Down syndrome had biparietal diameter/humerus length ratios >95th percentile, with the same seven being <90th percentile with no new cases identified. The relationship between humerus length and biparietal diameter is shown in Fig. 3 and is described by the linear equation humerus length = 1.146 biparietal diameter + 1.14 (r = 0.936, R^2 = 0.877, p = 0.0001). Five of 11 cases (45%) of Down syndrome had humerus length <5th percentile, with the sensitivity improving to seven of 11 (64%) if the 10th percentile was used as the cutoff. During the study period, six cases of trisomy 18 and no cases of trisomy 13 were identified. Only two of the six cases (sensitivity, 33%) of trisomy 18 had humerus length <5th percentile.

Abnormal femur length measurements were not as sensitive in detecting Down syndrome in comparison with humerus length. When femur length versus gestational age was considered, only two of 11 cases were below the 5th percentile and three of 11 below the 10th percentile. The biparietal diameter/femur length ratio versus gestational age (Fig. 4) identified two of 11 cases and three of 11 cases at the 95th and 90th percentile, respectively. The relationship between biparietal diameter/femur length and gestational age is described by the second-order polynomial equation biparietal diameter/femur length = 3.82 − 0.197 gestational age + 0.004 gestational age^2 (r = 0.59, R^2 = 0.348, p = 0.0001). Femur length versus gestational age identified only one of 11 and three of 11 cases at the 95th and 90th percentiles, respectively.

We concluded from these analyses that the humerus length versus gestational age was the simplest and most efficacious and thus the most practical ultrasonographic screening test for Down syndrome. The 5th percentile humerus length for gestational ages from 12 to 26 weeks is shown in Table I. Over the 2½-year study period, 1907 genetic amniocenteses were performed at the University of Connecticut Health Center (1431 cases) and Hartford Hospital (476 cases). Eleven cases of Down syndrome were identified in the 1907 cases, for an overall prevalence of 1 in 173. The sensitivity,

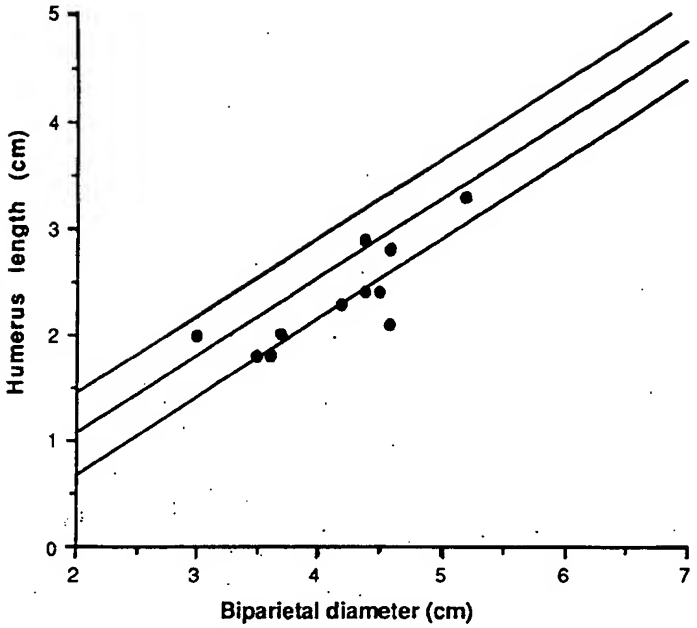

Fig. 3. Relationship between humerus length and biparietal diameter throughout second trimester. *Closed circles,* Fetuses with Down syndrome.

Table I. Cutoff values (5th percentile) for humerus length from 12 to 26 weeks' gestation.

Gestational age (completed weeks)	*Abnormally short (5th percentile) humerus length (mm)*
12	7
13	9
14	12
15	14
16	16
17	19
18	21
19	24
20	26
21	29
22	31
23	34
24	36
25	38
26	41

specificity, and positive and negative predictive value of humerus length versus gestational age is shown in Table II. An abnormally short femur length was not as sensitive as humerus length in detecting Down syndrome, as indicated in Table III. The positive predictive value of an abnormally short (<5th percentile) humerus length was 6.8% in our population of patients seen for genetic amniocentesis, who had a prevalence of Down syndrome of 1 in 173. Moreover, a normal humerus length measurement (≥5th percentile) would rule out Down syndrome in 99.7% of cases (negative predictive value).

Comment

Since 1970, very little has changed in regard to prenatal diagnosis of Down syndrome. The emphasis has been on testing women of advanced maternal age, although only 20% of Down syndrome occurs in that age group. Recently, maternal serum α-fetoprotein testing has been incorporated into the prenatal diagnostic testing schema, because women who are carrying fetuses with Down syndrome have been shown to have lower levels of maternal serum α-fetoprotein. If all women under 35 had routine maternal serum α-fetoprotein screening, another 20% of Down syndrome cases would be identified.[9] More recently, fetal ultrasonography has been suggested as an additional tool to aid in prenatal detection of Down syndrome. However, this method has not been very practical for the average clinician for several reasons. First, while some anomalies are frequently associated with Down syndrome they are not present in the majority of cases. For example, while 30% to 40% of fetuses with duodenal atresia prove to have Down syndrome, only 5% of fetuses with Down syndrome have duodenal atresia.[10] Second, some of the anomalies associated with Down syndrome are not readily detectable until the third trimester, when elective pregnancy termination is no longer an option for the parents. Duodenal atresia is one example, where the classic "double bubble" finding in association with polyhydramnios is not usually visualized before 24 weeks.[3] Third, some of the ultrasonographic findings may not be readily apparent or easy to obtain for the average sonographer on a routine examination.

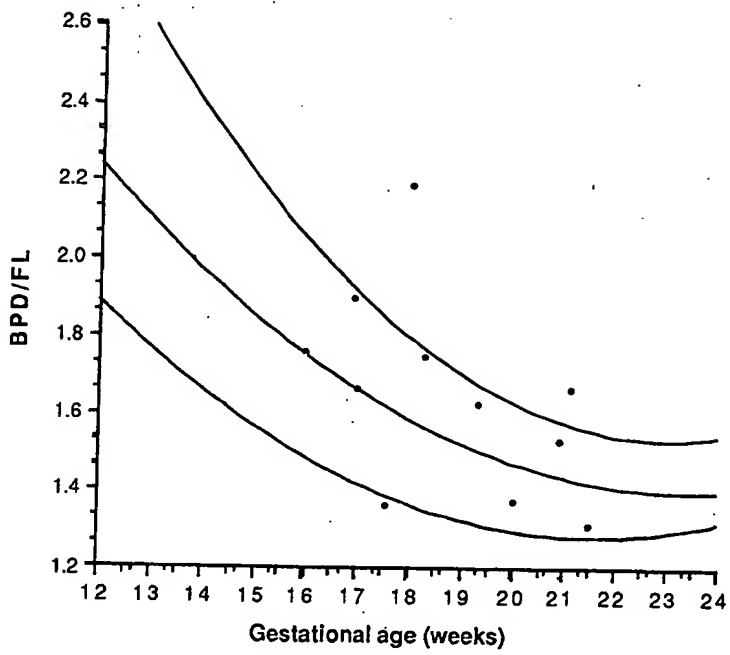

Fig. 4. Relationship between biparietal diameter/femur length *(BPD/FL)* ratio versus gestational age. Shown are 5th, 50th, and 90th percentiles. *Closed circles,* Fetuses with Down syndrome.

Examples include nuchal skin thickening, cardiac defects, and hypoplasia of the middle phalanx of the fifth finger.[11] Fourth, some mathematic calculations may be required, as in the case of biparietal diameter/femur length ratio. Moreover, the sensitivity of this ratio to detect Down syndrome has recently been questioned.[12]

Humerus length measurements, on the other hand, are easy to obtain. Long-bone measurements, particularly the femur length, are included in most, if not all, second-trimester ultrasonographic examinations. Including the humerus length as part of the routine examination requires no special expertise in obstetric ultrasonography and would add very little time to the examination. No ratios need to be calculated. In our population 64% of cases of Down syndrome would have been identified by this simple prenatal screening method, whereas <5% of normal fetuses had an abnormally short humerus length.

Our findings appear to agree with the recent observations of Benacerraf et al.[13] They found that if the actual-to-expected humerus length ratio was ≤0.90, they were able to detect 12 of 24 fetuses with Down syndrome (sensitivity, 50%) with a 6.25% false-positive rate.

It would appear that if our findings are confirmed, fetal humerus length measurements should be obtained at any second-trimester ultrasonographic examination. If the humerus length falls below the 5th percentile, prenatal genetic testing should be offered to the pa-

Table II. Efficacy of humerus length to predict Down syndrome

	Down syndrome	*Normal*	*Total*
Humerus length <5th percentile	7	95	102
Humerus length ≥5th percentile	4	1795	1799
TOTAL	11	1890	1901*

Sensitivity, 7 of 11 (64%); specificity, 1795 of 1890 (95%); positive predictive value, 7 of 102 (7%); negative predictive value, 1795 of 1799 (99.7%).

*The six cases of trisomy 18 were excluded.

Table III. Efficacy of femur length to predict Down syndrome

	Down syndrome	*Normal*	*Total*
Femur length <5th percentile	2	95	97
Femur length ≥5th percentile	9	1795	1804
TOTAL	11	1890	1901*

Sensitivity; 2 of 11 (18%); specificity; 1795 of 1890 (95%); positive predictive value; 2 of 97 (2%); negative predictive value; 1795 of 1804 (99.5%).

*The six cases of trisomy 18 were excluded.

tient. This may prove to be an effective way of identifying fetuses with Down syndrome in a low-risk population, specifically women <35 years old. We are prospectively evaluating the efficacy of an abnormal humerus length to predict Down syndrome in such low-risk women.

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Ultrasonographic diagnosis of congenital anomalies in twins

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Gainesville, Florida

To determine whether serial ultrasonographic examinations with basic anatomic surveys provide an adequate screen for congenital abnormalities that are more common in twins, we compared the results of prenatal sonograms and neonatal examinations for 314 twins (157 pairs) delivered during a recent 42-month period. An anomaly was defined as major if it potentially required surgical repair or precluded normal life expectancy; otherwise it was defined as minor. Thirty-three twins (9.5%) had 40 anomalies; 28 (9%) were major and 12 (4%) were minor. Prenatal ultrasonography with cardiac screening limited to the four-chamber view provided detection of 39% of all major anomalies, 55% of noncardiac major anomalies but none of the cardiac lesions, and 69% of the major anomalies for which routine prenatal management should be altered. No false-positive diagnoses incorrectly altered management. We conclude that serial prenatal ultrasonographic examinations are useful in detecting noncardiac anomalies for which twins are at increased risk, but the four-chamber view is not an adequate screen for the cardiac malformations of twins. (Am J Obstet Gynecol 1991;165:1056-60.)

Key words: Prenatal diagnosis, twins, ultrasonographic, congenital malformations

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Reprints not available.
6/6/30938

Twins are known to have an increased risk of congenital anomalies, up to threefold higher than for singletons[1-5]; however, the value of ultrasonography in the diagnosis and management of the anomalies of twins has not been proved. To determine whether ultrasonographic examinations that include a basic ana-

Exhibit E

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Femur and humerus length in trisomy 21 fetuses at 11–14 weeks of gestation

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KEYWORDS: chromosomal defect; femur length; first trimester; humerus length; nuchal translucency; screening; trisomy 21

ABSTRACT

Objective To determine the value of measuring fetal femur and humerus length at 11–14 weeks of gestation in screening for chromosomal defects.

Methods Femur and humerus lengths were measured using transabdominal ultrasound in 1018 fetuses immediately before chorionic villus sampling for karyotyping at 11–14 weeks of gestation. In the group of chromosomally normal fetuses, regression analysis was used to determine the association between long bone length and crown–rump length (CRL). Femur and humerus lengths in fetuses with trisomy 21 were compared with those of normal fetuses.

Results The median gestation was 12 (range, 11–14) weeks. The karyotype was normal in 920 fetuses and abnormal in 98, including 65 cases of trisomy 21. In the chromosomally normal group the fetal femur and humerus lengths increased significantly with CRL (femur length = −6.330 + 0.215 × CRL in mm, r = 0.874, P < 0.0001; humerus length = −6.240 + 0.220 × CRL in mm, r = 0.871, P < 0.0001). In the Bland–Altman plot the mean difference between paired measurements of femur length was 0.21 mm (95% limits of agreement −0.52 to 0.48 mm) and of humerus length was 0.23 mm (95% limits of agreement −0.57 to 0.55 mm). In the trisomy 21 fetuses the median femur and humerus lengths were significantly below the appropriate normal mean for CRL by 0.4 and 0.3 mm, respectively (P = 0.002), but they were below the respective 5th centile of the normal range in only six (9.2%) and three (4.6%) of the cases, respectively.

Conclusion At 11–14 weeks of gestation the femur and humerus lengths in trisomy 21 fetuses are significantly reduced but the degree of deviation from normal is too small for these measurements to be useful in screening for trisomy 21.

INTRODUCTION

Trisomy 21 is characterized by short stature and in the last decade several ultrasonographic studies have reported that during the second trimester the condition is associated with relative shortening of the femur and more so the humerus[1–5]. In the combined data from two leading centers of obstetric ultrasound, shortening of the femur was observed in 5.2% of 9331 normal fetuses and 41.4% of 319 trisomy 21 fetuses; the respective values for short humerus were 1.5% and 33.4%[6,7].

The aim of the present study was to determine the potential value of measuring fetal femur and humerus length at 11–14 weeks of gestation as a screening test for trisomy 21.

METHODS

We measured the fetal femur and humerus lengths at the routine ultrasound scan carried out before fetal karyotyping, by chorionic villus sampling (CVS), in 1018 consecutively examined fetuses at 11–14 weeks of gestation. There were 992 singleton pregnancies and 13 twin pregnancies in which each fetus was examined. The study was carried out in our center during a 5-month period (November 2002–March 2003). In all cases there was prior screening for chromosomal defects by a combination of maternal age and fetal nuchal translucency (NT) and the patients included in this study were those that after counseling elected to have invasive testing[8].

The fetal femur and humerus were examined by transabdominal sonography and the aim was for the angle between the ultrasound transducer and the bone

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examined to be about 45° since in this position a sharp image of the edges of the bones is obtained (Figure 1). Bone length was measured with calipers on the screen and the magnification of the image was such that each increment in the distance between calipers was only 0.1 mm. The fetal NT and crown–rump length (CRL) were also measured[8]. Examination of the fetal femur and humerus was successfully achieved in all cases and this added 1–3 min to the overall time of about 15 min for the 11–14-week scan.

Demographic characteristics and ultrasound findings were recorded in a fetal database at the time of the examination. In all cases CVS was carried out and when the fetal karyotype results were made available they were entered in the database also.

Statistical analysis

In the chromosomally normal group, regression analysis was used to determine the significance of the association between femur and humerus length with CRL. Each measurement of the long bones' length was then expressed as a difference from the expected mean for CRL (delta value) and the Mann–Whitney *U*-test was used to determine the significance of difference in the delta values between the chromosomally normal and trisomy 21 fetuses. Regression analysis was used to determine the significance of the association between delta femur and humerus length and delta NT thickness both in the chromosomally normal and trisomy 21 fetuses. In the 70 cases with paired measurements, the Bland–Altman plot (the difference between the two paired measurements versus the average of the two measurements) was performed and the 95% tolerance interval for paired observations was calculated[9].

RESULTS

The median maternal age was 37 (range, 17–48) years, the median fetal CRL was 65 (range, 45–84) mm and the median gestation was 12 (range, 11–14) weeks. The maternal ethnic group was Caucasian in 908 (89.2%) cases, Afro-Caribbean in 40 (3.9%), Asian in 39 (3.8%), Chinese or Japanese in 18 (1.8%) and mixed in 13 (1.3%). The fetal femur and humerus were successfully examined in all cases. The fetal karyotype was normal in 920 pregnancies and abnormal in 98, including 65 cases of trisomy 21 and 33 with other abnormalities (14 of trisomy 18, five of trisomy 13, two of trisomy 22, eight of Turner syndrome, two of Klinefelter syndrome, one of triploidy and one partial deletion of chromosome 3).

In the Bland–Altman plot the mean difference between paired measurements of femur length was 0.21 mm and the 95% limits of agreement were −0.52 to 0.48 mm (Figure 2). The respective values for humerus length were 0.23 mm (95% limits, −0.57 to 0.55 mm).

In the chromosomally normal group the fetal femur and humerus length increased significantly with CRL from respective means of 3.3 and 3.7 mm at CRL 45 mm to 11.9 and 12.5 mm at CRL 85 mm, respectively (femur length $= -6.330 + 0.215 \times$ CRL in mm, $r = 0.874$, $P < 0.0001$; humerus length $= -6.240 + 0.220 \times$ CRL in mm, $r = 0.871$, $P < 0.0001$; Figure 3).

In the trisomy 21 fetuses the median femur length was significantly below the normal mean for CRL by 0.386 mm (range, −2.583 to 2.132; $P = 0.002$). Similarly, the median humerus length was significantly below the appropriate normal mean for CRL by 0.338 mm (range, −2.174 to 2.007; $P = 0.002$). In the fetuses with other chromosomal abnormalities the median femur length was not significantly different from the normal mean for CRL (mean difference, 0.236 mm; range, −2.694 to 2.184; $P = 0.212$). Similarly, the median humerus length was not significantly different from the normal mean for CRL (mean difference, 0.078 mm; range, −2.233 to 1.861; $P = 0.582$). In the trisomy 21 fetuses the median femur and humerus lengths were below the respective 5th centile of the normal range in only six (9.2%) and three (4.6%) of the cases, respectively (Figure 4).

There was no significant association between the delta score of bone length and delta NT in either the chromosomally normal fetuses ($r = -0.061$, $P = 0.066$

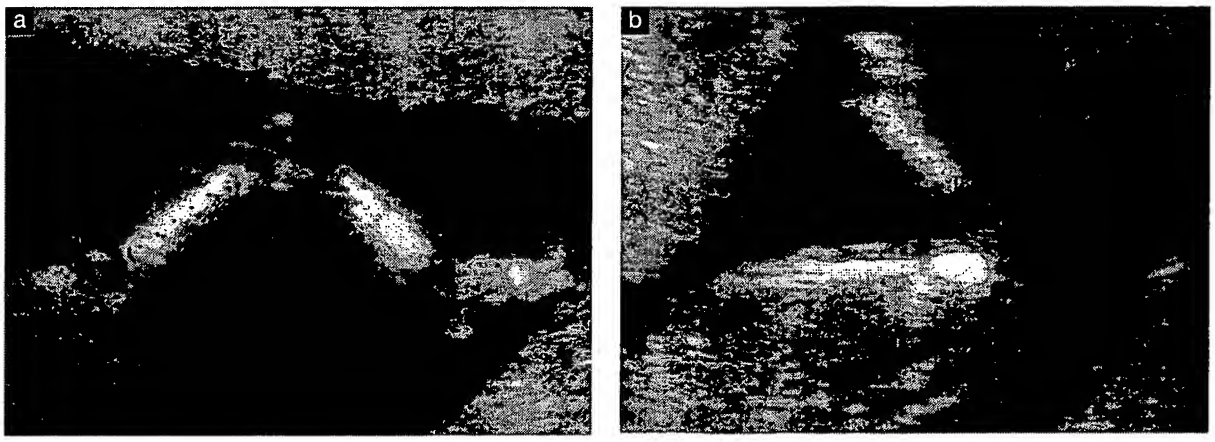

Figure 1 Ultrasound image of a 12-week fetus demonstrating measurement of (a) femur and (b) humerus length.

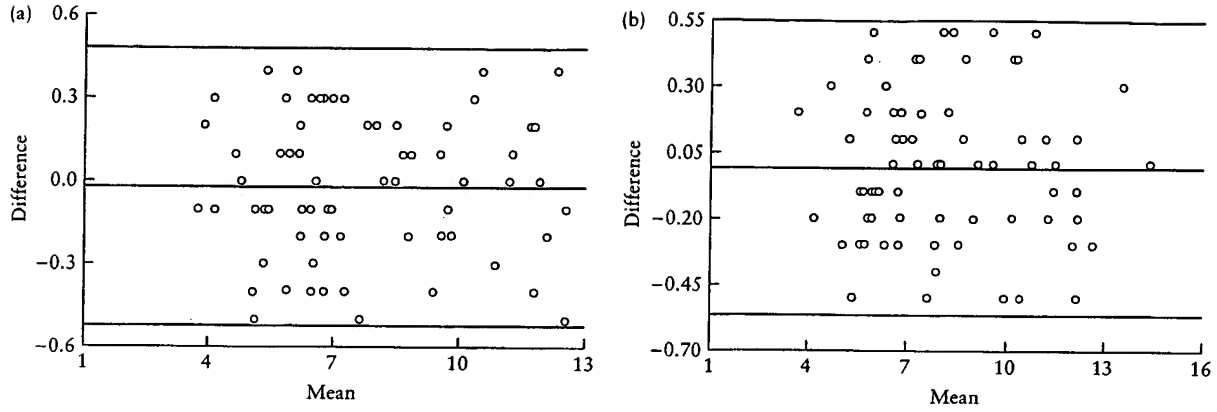

Figure 2 Bland–Altman plot of the difference against the mean of paired measurements in (a) femur and (b) humerus length.

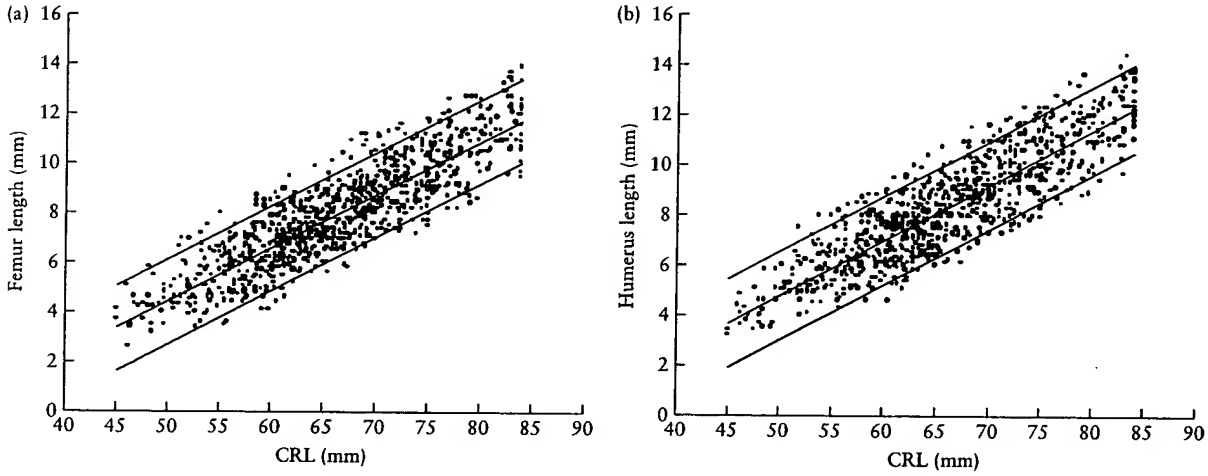

Figure 3 Reference range (mean, 95th and 5th centiles) with crown–rump length (CRL) in (a) femur and (b) humerus length in the chromosomally normal fetuses at 11–14 weeks of gestation.

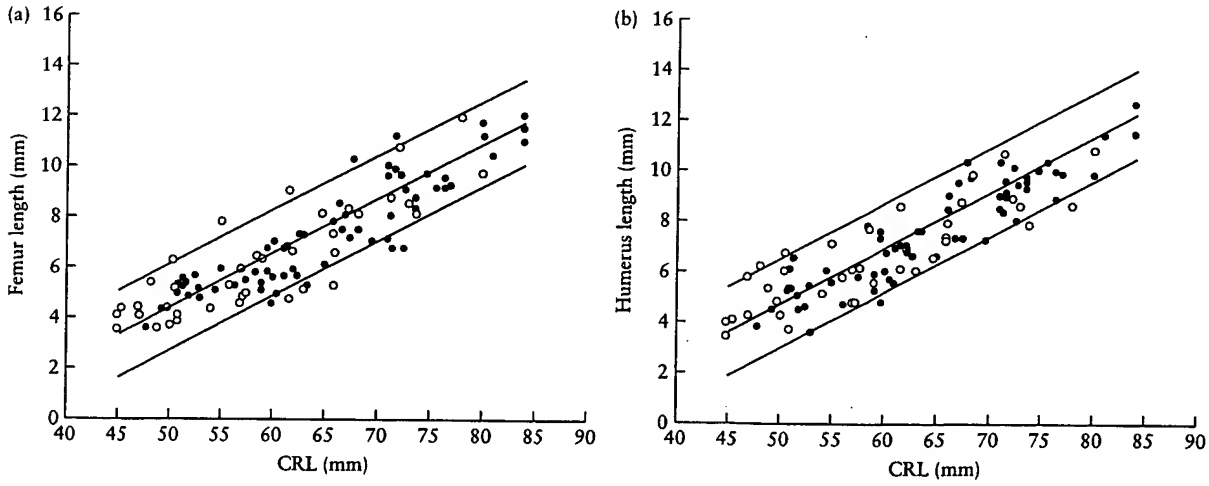

Figure 4 Fetal (a) femur and (b) humerus length in trisomy 21 (●) and other chromosomal defects (○) plotted on the reference range (mean, 95th and 5th centiles) with crown–rump length (CRL) of the chromosomally normal fetuses.

Table 1 Femur and humerus length at crown–rump lengths of 45 and 85 mm in the present study and previous reports on long bones in the first trimester

Study	Scan route	Femur length (mm)		Humerus length (mm)	
		CRL = 45 mm	CRL = 85 mm	CRL = 45 mm	CRL = 85 mm
Kustermann *et al.* (1992)[10]	Vaginal	6.9	13.5		
Zorzoli *et al.* (1994)[11]	Vaginal	6.3	13.1	5.3	13.1
Rosati and Guariglia (1997)[12]	Vaginal	6.0	15.6	5.6	15.0
Gabrielli *et al.* (1999)[13]	Vaginal	5.1	12.4		
von Kaisenberg *et al.* (2002)[14]	Abdominal	4.8	12.6		
Present study	Abdominal	3.3	11.9	3.7	12.5

*For the majority of the previous studies approximate values were extracted from the information provided. CRL, crown–rump length.

for femur length; $r = -0.022$, $P = 0.507$ for humerus length) or the trisomy 21 fetuses ($r = 0.017$, $P = 0.891$ for femur length; $r = -0.096$, $P = 0.445$ for humerus length).

DISCUSSION

This study has demonstrated the feasibility of measuring the fetal femur and humerus length at 11–14 weeks of gestation. These bones were successfully visualized and measured in all fetuses and the length of both bones increased linearly with gestation. The mean values of our measurements were shorter than those in all previous reports (Table 1)[10–14]. The most striking difference is observed in the long bone measurements at CRL of 45 mm with our measurements being about half of those reported in studies from the early 1990s. The most obvious explanations are that, first, the resolution and magnification of the image have improved during the last 10 years, allowing better visualization of the bones and more accurate placement of the calipers, and second, we ensured that the artifactual echogenicity beyond each end of the bones was minimized by maintaining a 45° angle between the ultrasound transducer and the bone examined. An additional problem with some of the previous studies is the gestational assessment of the fetuses examined. For example, in one study the mean CRL at 15 weeks is reported to be 85.5 mm, which is a substantial underestimate of the true measurement[12].

The finding that in trisomy 21 fetuses at 11–14 weeks of gestation the femur and humerus lengths were significantly reduced is compatible with the well-described association of trisomy 21 and shortening of the long bones in both postnatal studies and prenatal sonographic data from the second trimester of pregnancy[1–7]. Furthermore, there was no significant association between the degree of femur or humerus shortening and increase in NT.

The relative shortening of the femur and humerus of trisomy 21 fetuses may increase with gestation. For example, a study examining the biparietal diameter to femur length ratio reported that the ratio was above the 95th centile of the normal range in 24% of trisomy 21 fetuses at 18–20 weeks but in only 11% of cases at 15–17 weeks[15]. In the present study at 11–14 weeks the femur length was below the 5th centile of the normal range in only 9% of trisomy 21 fetuses. An additional problem with early gestation is the poor reproducibility of the measurements. Thus, at 11–14 weeks the mean difference between paired measurements was 0.21 mm for femur length and 0.23 for humerus length and the mean difference in femur and humerus length between trisomy 21 and normal fetuses was only 0.4 mm and 0.3 mm, respectively. Consequently, measurement of femur and humerus length at 11–14 weeks is unlikely to be useful in screening for trisomy 21.

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Exhibit F

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REVIEW ARTICLE

Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities

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Abstract: There is extensive evidence that effective screening for major chromosomal abnormalities can be provided in the first trimester of pregnancy. Prospective studies in a total of 200,868 pregnancies, including 871 fetuses with trisomy 21, have demonstrated that increased nuchal translucency can identify 76.8% of fetuses with trisomy 21, which represents a false-positive rate of 4.2%. When fetal nuchal translucency was combined with maternal serum free–β-human chorionic gonadotropin and pregnancy-associated plasma protein-A in prospective studies in a total of 44,613 pregnancies, including 215 fetuses with trisomy 21, the detection rate was 87.0% for a false-positive rate of 5.0%. Studies from specialist centers with 15,822 pregnancies, which included 397 fetuses with trisomy 21, have demonstrated that the absence of the nasal bone can identify 69.0% of trisomy 21 fetuses, which represents a false-positive rate of 1.4%. It has been estimated that first-trimester screening by a combination of sonography and maternal serum testing can identify 97% of trisomy 21 fetuses, which represents a false-positive rate of 5%, or that the detection rate can be 91%, which represents a false-positive rate of 0.5%. In addition to increased nuchal translucency, important sonographic markers for chromosomal abnormalities, include fetal growth restriction, tachycardia, abnormal flow in the ductus venosus, megacystis, exomphalos and single umbilical artery. Most pregnant women prefer screening in the first, rather than in the second, trimester. As with all aspects of good clinical practice, those care givers who perform first-trimester screening should be trained appropriately, and their results should be subjected to external quality assurance.

In 1866, Down[1] reported that, in individuals with trisomy 21 (the condition that came to bear his name), the skin appears to be too large for the body, the nose is small, and the face is flat. In the last decade, it has become possible to observe these features by ultrasound examination in the third month of intrauterine life. Approximately 75% of trisomy 21 fetuses have increased nuchal translucency (NT) thickness, and 70% of the fetuses have absent nasal bone.

During the last 30 years, extensive research has aimed at developing a noninvasive method for prenatal diagnosis of chromosomal and other abnormalities through the isolation and examination of fetal cells that are found in the maternal circulation. However, on the basis of currently available data,[2,3] there is no realistic prospect that, in the foreseeable future, noninvasive diagnosis will replace the need for invasive testing.

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Table I Estimated risk for trisomies 21, 18, and 13 (1 per number given in the table) in relation to maternal age and gestation[10-12]

	Trisomy 21				Trisomy 18				Trisomy 13			
	Gestation (wk)				Gestation (wk)				Gestation (wk)			
Maternal age (y)	12	16	20	40	12	16	20	40	12	16	20	40
20	1068	1200	1295	1527	2484	3590	4897	18013	7826	11042	14656	42423
25	946	1062	1147	1352	2200	3179	4336	15951	6930	9778	12978	37567
30	626	703	759	895	1456	2103	2869	10554	4585	6470	8587	24856
31	543	610	658	776	1263	1825	2490	9160	3980	5615	7453	21573
32	461	518	559	659	1072	1549	2114	7775	3378	4766	6326	18311
33	383	430	464	547	891	1287	1755	6458	2806	3959	5254	15209
34	312	350	378	446	725	1047	1429	5256	2284	3222	4277	12380
35	249	280	302	356	580	837	1142	4202	1826	2576	3419	9876
36	196	220	238	280	456	659	899	3307	1437	2027	2691	7788
37	152	171	185	218	354	512	698	2569	1116	1575	2090	6050
38	117	131	142	167	272	393	537	1974	858	1210	1606	4650
39	89	100	108	128	208	300	409	1505	654	922	1224	3544
40	68	76	82	97	157	227	310	1139	495	698	927	2683
41	51	57	62	73	118	171	233	858	373	526	698	2020
42	38	43	46	55	89	128	175	644	280	395	524	1516

Prenatal diagnosis requires either amniocentesis from 16 weeks of gestation or chorionic villous sampling from 11 weeks of gestation. Randomized studies have demonstrated that the procedure-related risk of miscarriage is the same (approximately 1%).[4-8] Consequently, invasive testing is carried out only in pregnancies that are considered to be at high risk for chromosomal abnormalities. The traditional method of screening is maternal age, with which invasive testing in 5% of the population identifies approximately 30% of the fetuses with trisomy 21. There is now extensive evidence that ultrasound examination, combined with maternal serum biochemical testing at 11 to 13 weeks of gestation, can identify >95% of the fetuses with major chromosomal abnormalities.

This article reviews the evidence on the association between chromosomal abnormalities and increased NT and other sonographic markers in the first trimester of pregnancy.

Methods

Searches of PubMed were made to identify all articles that have been published since 1990 on first trimester sonographic markers of chromosomal abnormalities. Most publications were on fetal NT, which were grouped into series that reported on the association between increased NT and chromosomal abnormalities, into series that reported on prospective screening studies with NT alone or NT in combination with first or second trimester maternal serum biochemical testing, and into series that reported on observational screening studies.

Patient-specific risk for chromosomal abnormalities

Every woman has a risk that her fetus/baby has a chromosomal defect. To calculate the individual risk, it is necessary to take into account the a priori risk, which depends on maternal age and gestational age, and to multiply this by a likelihood ratio, which depends on the results of ultrasound findings and/or maternal serum biochemical tests that were performed during the course of the pregnancy to determine the patient-specific risk.[9] Every time a test is carried out, the a priori risk is multiplied by the likelihood ratio that is derived from that test to calculate a new risk, which then becomes the a priori risk for the next test. This process of sequential screening necessitates that the different tests are independent of each other. If the tests are not independent of each other, then more sophisticated techniques that involve multivariate statistics can be used to calculate the combined likelihood ratio.

Maternal age and gestation

The risk for many of the chromosomal abnormalities increases with maternal age. Additionally, because fetuses with chromosomal abnormalities are more likely to die in utero than normal fetuses, the risk decreases with advancing gestation (Table I).[10-12] Estimates of the maternal age-related risk for trisomy 21 at birth are based on surveys with almost complete ascertainment of the affected patients.[13] During the last decade, with the introduction of maternal serum biochemistry and ultrasound screening for chromosomal abnormalities at

different stages of pregnancy, it has become necessary to establish maternal age and gestational age-specific risks for chromosomal abnormalities. Such estimates were derived by a comparison of the birth prevalence of trisomy 21 to the prevalence in women who undergo second-trimester amniocentesis or first-trimester chorionic villus sampling.

The rates of fetal death in trisomy 21 between 12 weeks of gestation (when NT screening is performed) and 40 weeks of gestation is approximately 30% and between 16 weeks of gestation (when second trimester serum biochemistry is performed) and 40 weeks of gestation is approximately 20%.[10-12,14,15] In trisomies 18 and 13, the rate of fetal death between 12 and 40 weeks of gestation is approximately 80% (Table I).[12] The frequency of conception of 45,X embryos, unlike that of trisomies, is unrelated to maternal age; and the prevalence is approximately 1 per 1500 fetuses at 12 weeks of gestation, 1 per 3000 fetuses at 20 weeks of gestation, and 1 per 4000 fetuses at 40 weeks of gestation. Polyploidy affects approximately 2% of recognized conceptions, but it is highly lethal and thus rarely observed in live births; the prevalence at 12 and 20 weeks of gestation is approximately 1 per 2000 fetuses and 1 per 250,000 fetuses, respectively.[12]

Fetal NT thickness

Cystic hygromas, nuchal edema, and NT

During the second and third trimesters of pregnancy, abnormal accumulation of fluid behind the fetal neck can be classified as nuchal cystic hygroma or nuchal edema.[16,17] In approximately 75% of fetuses with cystic hygromas, there is a chromosomal abnormality; in approximately 95% of cases, the abnormality is Turner syndrome.[18] Nuchal edema has a diverse cause; chromosomal abnormalities are found in approximately one third of the fetuses, and in approximately 75% of these cases, the abnormality is trisomy 21 or 18.[19] Edema also is associated with fetal cardiovascular and pulmonary defects, skeletal dysplasias, congenital infection, and metabolic and hematologic disorders; consequently, the prognosis for chromosomally normal fetuses with nuchal edema is poor.[19]

In the first trimester, the term *translucency* is used, irrespective of whether the collection of fluid is septated and whether it is confined to the neck or envelopes the whole fetus.[20] Cullen et al[21] examined 29 fetuses with abnormal nuchal fluid at 10 to 13 weeks of gestation and reported that neither the incidence of chromosomal abnormalities nor the prognosis could be predicted by the ultrasonographic appearance of the lesion. Increased NT is associated with trisomy 21, Turner syndrome, and other chromosomal abnormalities as well as many fetal malformations and genetic syndromes.[22,23] The prevalence of these abnormalities is related to the thickness, rather than the appearance, of NT. Furthermore, it is possible to standardize and audit the results of a measurement but not those of a subjective appearance.

Pathophysiologic evidence of increased NT

Increased fetal NT is associated with a wide range of chromosomal and other abnormalities.[22,23] The heterogeneity of conditions suggests that there may not be a single underlying mechanism for the collection of fluid in the skin of the fetal neck. Possible mechanisms include cardiac failure in association with abnormalities of the heart and great arteries;[24-27] venous congestion in the head and neck caused by constriction of the fetal body in amnion rupture sequence or superior mediastinal compression found in diaphragmatic hernia or the narrow chest in skeletal dysplasia;[22,28,29] altered composition of the extracellular matrix that may be attributed to gene dosage effects;[30,31] abnormal or delayed development of the lymphatic system;[32,33] failure of lymphatic drainage because of impaired fetal movements in various neuromuscular disorders;[34,35] fetal anemia or hypoproteinemia;[36-39] or congenital infection that acts through anemia or cardiac dysfunction.[40-42]

In fetuses with increased NT, the risk of an adverse outcome, which includes chromosomal and other abnormalities and fetal and postnatal death, increases with NT thickness from approximately 5% for NT between the 95th percentile and 3.4 mm to 30% for NT of 3.5 to 4.4 mm to 50% for NT of 4.5 to 5.4 mm and 80% for NT of $\geq$ 5.5 mm.[22,23] In most cases with increased fetal NT, a series of antenatal investigations that includes fetal karyotyping, detailed scans, fetal echocardiography, and genetic testing and infection screening can be completed by 20 weeks of gestation and will distinguish between the pregnancies that are destined to result in adverse outcome and the pregnancies that are destined to the delivery of infants without major defects.[23]

Measurement of NT

The ability to achieve a reliable measurement of NT is dependent on appropriate training and adherence to a standard technique to achieve uniformity of results among different operators.

Gestation and crown-rump length

The optimal gestational age for the measurement of fetal NT is 11 weeks of gestation to 13 weeks 6 days of gestation. The minimum fetal crown-rump length should be 45 mm, and the maximum length should be 84 mm.

The reasons for selecting 13 weeks 6 days of gestation as the upper limit are (1) to provide women who have

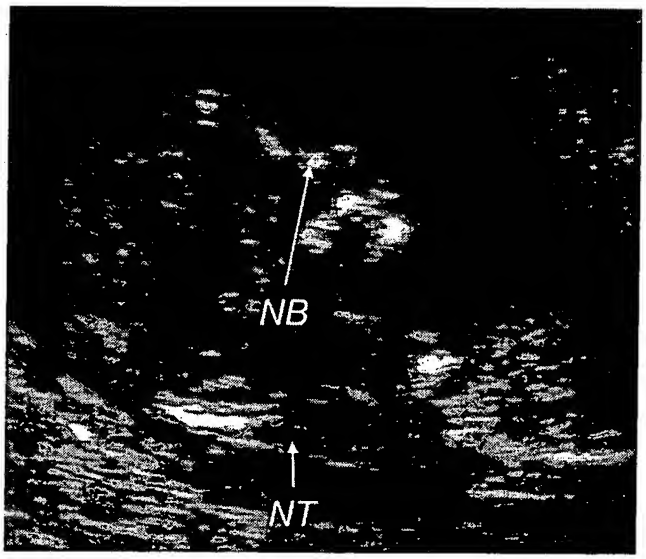

Figure 1 Ultrasound picture of a 12-week chromosomal fetus with normal NT thickness and a present nasal bone.

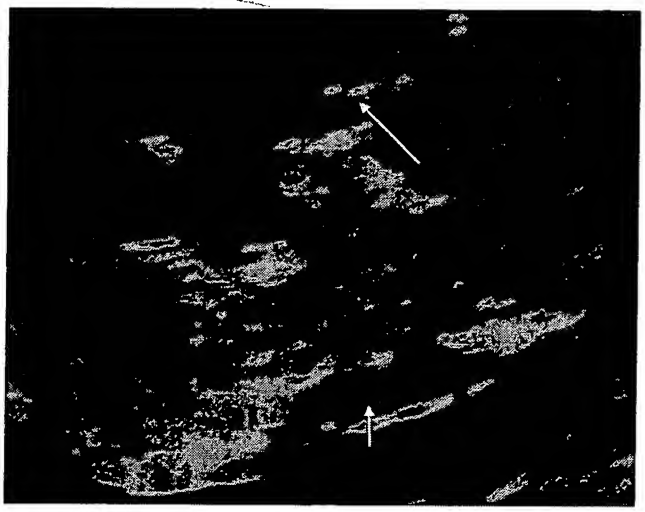

Figure 2 Ultrasound picture of a 12-week trisomy 21 fetus with increased NT thickness and an absent nasal bone.

affected fetuses the option of an earlier and safer form of termination; (2) the incidence of abnormal accumulation of nuchal fluid in chromosomally abnormal fetuses is lower at 14 to 18 weeks of gestation than at <14 weeks of gestation,[17,19,20,43] and (3) the success rate for taking a measurement at 10 to 13 weeks of gestation is 98% to 100%, which falls to 90% at 14 weeks of gestation because the fetus is often in a vertical position, which makes it more difficult to obtain the appropriate image.[44,45]

The reason for selecting 10 weeks of gestation as the earliest gestation was that screening necessitates the availability of a diagnostic test, and in the early 1990s it was appreciated that chorionic villous sampling before 10 weeks of gestation was associated with transverse limb reduction defects.[46,47] It was realized subsequently that many major fetal abnormalities could be diagnosed at the NT scan, if the minimum gestation is 11 weeks. For example, diagnosis or exclusion of acrania and therefore anencephaly cannot be made before 11 weeks of gestation because sonographic assessment of ossification of the fetal skull is not reliable before this gestation.[48] An examination of the 4-chamber view of the heart and main arteries is possible only after 10 weeks of gestation.[49-52] At 8 to10 weeks of gestation, all fetuses demonstrate herniation of the mid gut that is visualized as a hyperechogenic mass in the base of the umbilical cord; therefore, it is unsafe to diagnose or exclude exomphalos at this gestation.[53-55] The fetal bladder can be visualized in only 50% of fetuses at 10 weeks of gestation but in all cases by 12 weeks of gestation.[52,56,57]

In women who did not have a previous scan to date the pregnancy, it would be better to schedule the NT scan at 12 to 13 weeks of gestation, rather than at 11 weeks of gestation, because at this gestation some fetuses would be found to be too small; and a further scan would be necessary.[45]

Image and measurement

In the assessment of fetal NT, the ultrasound machine should be of high resolution with a video-loop function and calipers that provide measurements to 1 decimal point. Fetal NT can be measured successfully by transabdominal ultrasound examination in approximately 95% of cases; in the others, it is necessary to perform transvaginal sonography. The results from transabdominal and transvaginal scanning are similar.[58]

Only the fetal head and upper thorax should be included in the image for measurement of NT (Figures 1 and 2). The magnification should be as large as possible and always such that each slight movement of the calipers produces only a 0.1-mm change in the measurement. In the magnification of the image, either before or after the freeze zoom, it is important to turn the gain down. This avoids the mistake of placing the caliper on the fuzzy edge of the line, which causes an underestimate of the nuchal measurement.[59] A study in which rat heart ventricles were measured initially by ultrasound and then by dissection demonstrated that ultrasound measurements can be accurate to the nearest 0.1 to 0.2 mm.[60]

A good sagittal section of the fetus, as for measurement of fetal crown-rump length, should be obtained; and the NT should be measured with the fetus in the neutral position.[15] Hyperextension of the fetal neck can increase the NT measurement artificially by 0.6 mm, and flexion can decrease the measurement by 0.4 mm.[61]

Care must be taken to distinguish between fetal skin and amnion, because at this gestation both structures

appear as thin membranes (Figure 1).[20] This is achieved by waiting for spontaneous fetal movement away from the amniotic membrane; alternatively, the fetus is bounced off the amnion by asking the mother to cough and/or by tapping the maternal abdomen.

The maximum thickness of the subcutaneous translucency between the skin and the soft tissue overlying the cervical spine should be measured.[20] The calipers should be placed on the lines that define the NT thickness; the crossbar of the caliper should be such that it is hardly visible as it merges with the white line of the border and not in the nuchal fluid. During the scan, >1 measurement must be taken, and the maximum measurement should be used for the risk assessment.

The umbilical cord may be around the fetal neck in 5% to 10% of cases, which may produce a falsely increased NT and add approximately 0.8 mm to the measurement.[62,63] In such cases, the measurements of NT above and below the cord are different, and in the calculation of risk, it is more appropriate to use the average of the 2 measurements.[63]

There are no clinically relevant effects on NT measurements by ethnic origin,[64,65] parity or gravidity,[66] cigarette smoking,[67,68] diabetic control,[69] conception by assisted reproduction techniques,[70-73] bleeding in early pregnancy[74] or fetal gender.[75-77]

The intraobserver and interobserver differences in measurements of fetal NT are <0.5 mm in 95% of cases.[78-80]

Deviation in measurement from normal

Fetal NT increases with crown-rump length; therefore, it is essential to take gestation into account when a determination is made about whether a given NT thickness is increased.[59,81] In a study that involved 96,127 pregnancies, the median and 95th percentile at a crown-rump length of 45 mm were 1.2 and 2.1 mm; the respective values at a crown-rump length of 84 mm were 1.9 and 2.7 mm.[82] The 99th percentile did not change with crown-rump length and was approximately 3.5 mm.

In a screening for chromosomal abnormalities, patient-specific risks are derived by the multiplication of the a priori maternal age and gestation-related risk by a likelihood ratio, which depends on the difference in fetal NT measurement from the expected normal median for the same crown-rump length (Delta value).[81,82] In screening that uses maternal serum biochemical markers, a different approach has been used to take into account the gestational age-related change in marker levels. This method involves the conversion of the measured concentration into a multiple of the median (MoM) of unaffected pregnancies at the same gestation.[83] Essentially, the Gaussian distributions of $\log_{10}$ (MoM) in trisomy 21 and unaffected pregnancies are derived, and the heights of the distributions at a particular MoM, which is the likelihood ratio for trisomy 21, is used to modify the a priori maternal age-related risk to derive the patient-specific risk.

A study that involved the analysis of data of NT and crown-rump length from 128,030 unaffected and 428 trisomy 21 pregnancies demonstrated that the Delta NT approach provides accurate patient-specific risks.[84] In contrast, the MoM approach was found to be inappropriate for this purpose, because none of the 3 basic assumptions that underpin this method are valid. First, in the unaffected population, the distributions of NT MoM and $\log_{10}$ (NT MoM) were not Gaussian; second, the standard deviations did not remain constant with gestation; and third, the median MoM in the trisomy 21 pregnancies was not a constant proportion of the median for unaffected pregnancies. The MoM approach resulted in women being given an overestimate of risk for trisomy at 11 weeks of gestation and a considerable underestimate of risk at 13 weeks of gestation.

Training and quality assessment in the measurement of NT

Appropriate training of sonographers and adherence to a standard technique for the measurement of NT are essential prerequisites for good clinical practice. Furthermore, the success of a screening program necessitates the presence of a system for regular audit of results and continuous assessment of the quality of images. In 1997, a study group of the Royal College of Obstetricians and Gynaecologists in the United Kingdom recommended that NT screening should only be conducted by highly competent sonographers who are certified by an external agency and subject to external quality assurance and ongoing audit.[85]

All sonographers who perform fetal scans should be capable of reliably measuring the crown-rump length and obtaining a proper sagittal view of the fetal spine. For such sonographers, it is easy to acquire the skill to measure NT thickness within a few hours. However, the ability to measure NT and to obtain reproducible results improves with training. Good results are achieved after 80 scans for the transabdominal route and after 100 scans transvaginally.[86]

The Fetal Medicine Foundation (FMF), which is a UK registered charity, has established a process of training and quality assurance for the appropriate introduction of NT screening into clinical practice.[87] Training is based on a theoretic course and practical instruction on how to obtain the appropriate image and make the correct measurement of NT. The trainee is required subsequently to submit a logbook of images, which are examined to determine whether the magnification

Table II Early reports on the association between increased fetal NT thickness and chromosomal abnormalities

Study/year	NT thickness (mm)	N	Abnormal karyotype (n)					
			Total	Trisomy 21	Trisomy 18	Trisomy 13	45, X	Other
Johnson et al/1993[92]	≥ 2.0	68	41 (60.3%)	16	9	2	9	5
Hewitt/1993[93]	≥ 2.0	29	12 (41.4%)	5	3	1	2	1
Shulman et al/1992[94]	≥ 2.5	32	15 (46.9%)	4	4	3	4	—
Nicolaides et al/1992[20]	≥ 3.0	88	33 (37.5%)	21	8	2	—	2
Ville et al/1992[95]	≥ 3.0	29	8 (27.6%)	4	3	1	—	—
Wilson et al/1992[96]	≥ 3.0	14	3 (21.4%)	—	—	—	1	2
Trauffer et al /994[97]	≥ 3.0	43	21 (48.8%)	9	4	1	4	3
Brambati et al/1995[98]	≥ 3.0	70	8 (11.4%)	?	?	?	?	?
Comas et al/1995[99]	≥ 3.0	51	9 (17.6%)	4	4	—	—	1
Pandya et al/1995[100]	≥ 3.0	1,015	194 (19.1%)	101	51	13	14	15
Szabo et al/1995[101]	≥ 3.0	96	43 (44.8%)	28	10	—	2	3
Shulte-Valentin and Schindler/1992[102]	≥ 4.0	8	7 (87.5%)	7	—	—	—	—
van Zalen-Sprock et al/1992[1037]	≥ 4.0	18	6 (33.3%)	3	1	—	1	1
Nadel et al/1993[104]	≥ 4.0	63	43 (68.3%)	15	15	1	10	2
Savoldelli et al/1993[105]	≥ 4.0	24	19 (79.2%)	15	2	1	1	—
Cullen et al/1990[21]	≥ 6.0	29	15 (51.7%)	6	2	—	4	3
Suchet et al/1992[106]	≥ 10.0	13	8 (61.5%)	—	—	—	7	1
Total		1690	485 (28.7%)	238	116	25	59	39

is adequate, whether the section of the fetus is truly sagittal, whether the head is in the neutral position, whether the amnion is seen separately from the nuchal membrane and the calipers are placed appropriately. Ongoing quality assurance is based on an assessment of the distribution of fetal NT measurements and an examination of a sample of images that are obtained by each sonographer who is involved in screening. The distribution of measurements from each sonographer and each center is compared with those that were established by a major multicenter study co-ordinated by the FMF.[82] The services of the FMF, including certification, software for calculation of risk, and quality assurance are provided free-of charge.

Three studies have demonstrated that an ongoing regular audit of images and the distribution of measurements of NT are essential for the assessment of the quality of a center and are useful in the identification of individual sonographers whose results deviate from the mean performance.[88-90] The variation in measurements is reduced considerably after an initial learning phase and after feedback to the sonographers.

Additional evidence in favor of appropriate training of sonographers and adherence to a standard technique for the measurement of NT is provided by Monni et al,[91] who reported that, by modifying their technique of measuring NT in accordance with the guidelines that were established by the FMF, their detection rate of trisomy 21 improved from 30% to 84%.

The process of training, certification, and quality assurance in NT measurement, as introduced by the FMF, has been endorsed and is now being carried out by the national societies of obstetricians and gynecologists in Australia, Austria, Cyprus, Germany, and Italy. Similar systems are being developed in many other countries, which includes the United States, in collaborations between the FMF and local professional organizations.

NT thickness and risk for chromosomal abnormalities

In the early 1990s, several reports demonstrated that increased fetal NT thickness is associated with a high incidence of trisomy 21 and other chromosomal abnormalities (Table II).[20,21,92-106] In the combined data from 17 series that involved a total of 1690 patients, the incidence of chromosomal abnormalities was 28.7%. However, there were large differences between the studies in the incidence of chromosomal abnormalities, which ranged from 11% to 88%, because of differences in the maternal age distributions of the populations that were examined and the definition of the minimum abnormal NT thickness, which ranged from 2 to 10 mm.

Estimate of risk for trisomy 21 by maternal age and fetal NT

Studies in the mid 1990s demonstrated that (1) the risk of chromosomal abnormalities increases with both maternal age and fetal NT thickness and (2) in pregnancies with low fetal NT, the maternal age-related risk is decreased.[20,100,107,108] A study of 1015 pregnancies with increased fetal NT reported that the observed numbers of trisomies 21, 18, and 13 in fetuses with NT

Table III Prospective screening studies for trisomy 21 by measurement of fetal NT thickness

Study/year	Gestation (wk)	N	Successful measurement (%)	NT cut-off	False-positive rate (%)	Detection rate of trisomy 21 (n/N)
Pandya et al/1995[109]	$10\text{-}13^{+6}$	1,763	100.0	2.5 mm	3.4	3/4 (75.0%)
Schwarzler et al/1999[110]	$10\text{-}13^{+6}$	4,523	100.0	2.5 mm	2.7	8/12 (66.7%)
Schuchter et al/2001[111]	$10\text{-}12^{+6}$	9,342	100.0	2.5 mm	2.1	11/19 (57.9%)
Wayda et al/2001[112]	$10\text{-}13^{+0}$	6,841	100.0	2.5 mm	4.1	17/17 (100.0%)
Panburana et al/2001[113]	$10\text{-}13^{+6}$	2,067	100.0	2.5 mm	2.9	2/2 (100.0%)
Snijders et al/1998[82]	$10\text{-}13^{+6}$	96,127	100.0	95th percentile	4.4	234/326 (71.8%)
Theodoropoulos et al/1998[114]	$10\text{-}13^{+6}$	3,550	100.0	95th percentile	2.3	10/11 (90.9%)
Zoppi et al/2001[115]	$10\text{-}13^{+6}$	10,111	100.0	95th percentile	5.1	52/64 (81.3%)
Gasiorek-Wiens et al/2001[116]	$10\text{-}13^{+6}$	21,959	100.0	95th percentile	8.0	174/210 (82.9%)
Brizot et al/2001[117]	$10\text{-}13^{+6}$	2,492	100.0	95th percentile	6.4	7/10 (70.0%)
Comas et al/2002[43]	$10\text{-}13^{+6}$	7,345	100.0	95th percentile	4.9	38/38 (100.0%)
Chasen et al/2003[118]	$11\text{-}13^{+6}$	2,248	100.0	95th percentile	3.4	9/12 (75.0%)
Szabo et al/1995[119]	$9\text{-}12^{+6}$	3,380	100.0	3.0 mm	1.6	27/30 (90.0%)
Taipale et al/1997[120]	$10\text{-}13^{+6}$	6,939	98.6	3.0 mm	0.7	4/6 (66.7%)
Pajkrt et al/1998[121,122]	$10\text{-}13^{+6}$	3,614	100.0	3.0 mm	4.2	32/46 (69.6%)
Audibert et al/2001[123]	$10\text{-}13^{+6}$	4,130	95.5	3.0 mm	1.7	7/12 (58.3%)
Rosenberg et al/2002[124]	$12\text{-}14^{+0}$	6,234	98.6	3.0 mm	2.8	13/21 (61.9%)
Economides et al/1998[125]	$11\text{-}14^{+6}$	2,256	100.0	99th percentile	0.4	6/8 (75.0%)
Whitlow et al/1999[126]	$11\text{-}14^{+6}$	5,947	100.0	99th percentile	0.7	15/23 (65.2%)
Total		200,868	99.8		4.2	669/871 (76.8%)

In some of the studies, a cut-off in NT was used to define the screen-positive group; in other studies, the FMF software was used to estimate patient-specific risks that were based on maternal age, gestational age and fetal NT (Table IV).

of 3 mm, 4 mm, 5 mm, and >6 mm were approximately 3 times, 18 times, 28 times, and 36 times higher than the respective number expected on the basis of maternal age.[100] The incidences of Turner syndrome and triploidy were 9 times and 8 times higher; the incidence of other sex chromosome aneuploidies was similar to that expected.[100]

Subsequently, a prospective multicenter screening study at 10 to 14 weeks of gestation in 20,804 pregnancies, which included 164 cases of chromosomal abnormalities, demonstrated that, in normal pregnancies, fetal NT thickness increases with gestation; that, in trisomy 21 and other major chromosomal abnormalities, fetal NT is increased; and that the risk for trisomies can be derived by the multiplication of the a priori maternal age and gestation-related risk by a likelihood ratio, which depends on the degree of deviation in fetal NT measurement from the expected normal median for that crown-rump length.[81] It was estimated that, in a pregnant population with a mean maternal age of 28 years, a risk cut-off of 1 in 300 pregnancies that was used to define the screen positive group would detect approximately 80% of trisomy 21 fetuses, which represents a false positive rate of 5%.[81]

Implementation of NT screening in routine practice

Several prospective interventional studies have examined the implementation of NT screening in routine practice; the results are summarized in Tables III and IV.[43,82,109-130] In some of the studies, the screen-positive group was defined by a cut-off in fetal NT (Table III) or a combined risk that was derived from the maternal age and the deviation in fetal NT from the normal median for fetal crown-rump length (Table IV).

The important findings of these studies are that (1) fetal NT was measured successfully in >99% of cases; (2) there were inevitable variations in false-positive and detection rates between the studies because of differences in the maternal age distribution of their populations and in the fetal NT or risk cut-offs that were used; and (3) in the combined data on >200,000 pregnancies, which including >900 fetuses with trisomy 21, fetal NT screening identified >75% of fetuses with trisomy 21 and other major chromosomal abnormalities, which represents a false-positive rate of 5%, or the detection rate was approximately 60%, which represents a false-positive rate of 1% (Tables III and IV).[43,82,109-130]

In the study that was co-ordinated by the FMF, 100,311 singleton pregnancies were examined by 306 appropriately trained sonographers in 22 UK centers.[82] In all cases, the fetal NT and crown-rump length were measured, and individual patient-specific risks that were based on maternal age, gestational age, and fetal NT were calculated. Follow-up was obtained from 96,127 cases, which included 326 cases with trisomy 21 and 325 cases with other chromosomal abnormalities (Table V). The median gestation at the time of screening was 12 weeks (range, 10-14 weeks of gestation), and the median

Table IV Prospective screening studies for trisomy 21 at 10 to 14 weeks of gestation with the use of the FMF software to estimate patient-specific risks that were based on maternal age, gestational age and fetal NT thickness

Study/year	Mean maternal age (y)	Screen positive (n/N)			
		Cut-off	Normal	Chromosomal abnormalities	
				Trisomy 21	Other
Snijders et al/1998[82]	31	1/300	7,907/95,476 (8.3%)	268/326 (82.2%)*	253/325 (77.8%)
Theodoropoulos et al/1998[114]	29	1/300	151/3,528 (4.3%)	10/11 (90.9%)	11/11 (100.0%)
Thilaganathan et al/1999[127]	29	1/300	762/9,753 (7.8%)	17/21 (81.0%)*	25/28 (89.3%)
Schwarzler et al/1999[110]	29	1/270	212/4,500 (4.7 %)	10/12 (83.3%)	8/11 (72.7%)
O'Callaghan et al/2000[128]	32	1/300	59/989 (6.0%)	6/8 (75.0%)	3/3 (100.0%
Brizot et al/2001[117]	28	1/300	183/2,470 (7.4%)	9/10 (90.0%)	9/12 (75.0%)
Gasiorek-Wiens et al/2001[116]	33	1/300	2800/21,475 (13.0%)	184/210 (87.6%)	239/274 (88.2%)
Sau et al/2001[129]	28	1/100	61/2,600 (2.3%)	8/8 (100%)	5/7 (71.4%)
Zoppi et al/2001[115]	33	1/300	887/10,001 (8.9%)	58/64 (90.6%)	39/46 (84.8%)
Prefumo and Thilaganathan/2002[130]	31	1/300	565/11,820 (4.8%)	22/27 (81.5%)	—
Chasen et al/2003[118]	33	1/300	169/2,216 (7.5%)	10/12 (83.3%)*	15/20 (75.0%)
Total			13,756/164,828 (8.3%)	602/709 (84.9%)	607/737 (82.4%)

* In 3 studies, the detection rate at a fixed 5% false-positive rate was estimated. In the combined data on a total of 359 cases of trisomy 21, it was estimated that 278 cases (78.4%) would have been detected.

Table V A multicenter study that was co-ordinated by the FMF

Fetal karyotype	N	NT > 95th percentile	Risk ≥ 1 in 300
Normal	95,476	4209 (4.4%)	7907 (8.3%)
Trisomy 21	326	234 (71.2%)	268 (82.2%)
Trisomy 18	119	89 (74.8%)	97 (81.5%)
Trisomy 13	46	33 (71.7%)	37 (80.4%)
Turner syndrome	54	47 (87.0%)	48 (88.9%)
Triploidy	32	19 (59.4%)	20 (62.5%)
Other*	64	41 (64.1%)	51 (79.7%)
Total	96,127	4767 (5.0%)	8428 (8.8%)

Data are given as the number of pregnancies with NT thickness > 95th percentile and an estimated risk for trisomy 21 that was based on maternal age, fetal NT, and crown-rump length of 1 in ≥ 300.[82]

* Deletions, partial trisomies, unbalanced translocations, sex chromosome aneuploidies.

maternal age was 31 years. The estimated risk for trisomy 21 was >1 in 300 cases in 7907 (8.3%) of the normal pregnancies, in 268 cases (82.2%) of those with trisomy 21, and in 253 cases (77.8%) with other chromosomal abnormalities. For a screen-positive rate of 5%, the detection rate was 77% (95% CI, 72%-82%).[82]

The issue of fetal lethality

Screening for chromosomal abnormalities in the first, rather than the second, trimester has the advantage of earlier prenatal diagnosis and consequently less traumatic termination of pregnancy for those couples who choose this option. A potential disadvantage is that earlier screening preferentially identifies those chromosomally abnormal pregnancies that are destined to miscarry. Approximately 30% of affected fetuses die between 12 weeks of gestation and term.[10-12,14,15] This issue of preferential intrauterine lethality of chromosomal abnormalities is, of course, a potential criticism of all methods of antenatal screening, which include second-trimester maternal serum biochemistry, because the rate of intrauterine lethality between 16 weeks of gestation and term is approximately 20%.[10-12,14,15]

In a study of 109 fetuses with trisomy 21 that were diagnosed in the first trimester because of increased fetal NT, the parents chose to continue with the pregnancy in 6 cases, whereas in 103 cases the parents opted for termination.[131] In 5 of the 6 fetuses, the translucency resolved, and at the second-trimester scan, the nuchal-fold thickness was normal. All 6 trisomy 21 babies were born alive, but 1 baby had a major atrioventricular septal defect and died at the age of 6 months. These data suggest that increased NT does not identify necessarily those trisomic fetuses who are destined to die in utero.

In prenatal screening studies, it is impossible to know how many of the trisomy 21 pregnancies that were terminated would have resulted in live births. However, it is possible to estimate the impact of prenatal screening on the prevalence of trisomy 21 in live births. This can be done by a comparison of the number of affected live births with the number that are estimated on the basis of the maternal age-related prevalence of trisomy 21 in live births and the maternal age distribution of the population that was screened. In the FMF screening study, by a combination of maternal age and fetal NT, a risk cut-off of 1 in 300 was associated with a false-positive rate of 8.3% and a detection rate of 82.2%.[82] It was estimated

Table VI Results of observational studies on the effectiveness of NT providing data about gestation at screening, the number of patients who were recruited, and the number of women with satisfactory measurements of NT, false-positive rate, and detection rate of trisomy 21

Study/year	Gestation (wk)	N	Successful measurement (%)	NT cut-off	False-positive rate (%)	Detection rate (n/N)
Roberts et al/1995[132] Bewley et al/1995[133]	$8\text{-}13^{+6}$	1,704	66.1	3.0 mm	6.2	1/3 (33.3%)
Kornman et al/1996[134]	$8\text{-}13^{+6}$	923	58.2	3.0 mm	6.3	2/4 (50.0%)
Haddow et al/1998[135]	$9\text{-}15^{+6}$	4,049	83.0	95th percentile	5.0	18/58 (31.0%)
Crossley et al/2002[136]	$10\text{-}14^{+6}$	17,229	72.9	95th percentile	5.0	18/37 (48.6%)
Wald et al/2003[137]	$6\text{-}16^{+6}$	47,053	76.6*	95th percentile	5.0	29/75 (38.7%)
Total		70,958	75.1		5.0	68/177 (38.4%)

* Satisfactory images at 10 to14 weeks of gestation.

that prenatal screening followed by invasive diagnostic testing and selective termination of affected fetuses would have reduced the potential live birth prevalence of trisomy 21 by 78% to 82%.[82]

Observational studies

The ability to achieve a reliable measurement of NT is dependent on appropriate training, adherence to a standard technique, and motivation of the sonographer. All 3 components are well illustrated by the differences in results between interventional (Tables III and IV) and observational studies, in which the sonographers were asked to record the fetal NT measurements but not act on the results (Table VI).[132-137] Thus, successful measurement of NT was achieved in >99% of cases in the interventional studies (Table III), but in only 75% of cases in the observational studies (Table VI). Furthermore, in the interventional studies, there was increased NT in 76.8% of the trisomy 21 and 4.2% of the chromosomally normal fetuses, compared with the respective rates of 38.4% and 5.0% in the observational studies.

In the observational studies, the scans often were carried out at inappropriate gestations, and the sonographers were either not trained adequately or were not motivated sufficiently to measure NT. In the first study, the sonographers were instructed to take no extra scanning time other than that that was necessary for the measurement of the crown-rump length.[132] Fetal NT was measured successfully in only 66% of cases. In the second study, the fetal crown-rump length was <33 mm in 54% of cases, and the sonographers who were instructed to measure fetal NT within 3 minutes were unable to do so in 42% of cases.[134] In the third study in 16 centers, the sonographers did not receive any training but were given written instructions on how to measure NT.[135] Inevitably, there were large variations between centers in the ability to measure NT (median, 83%; range, 61%-100%), the median value of NT (median, 1.5 mm; range, 1.0-4.0 mm), and the percentage of trisomy 21 fetuses with NT at >95th percentile (median, 31%; range, 0-100%). The authors concluded that it is necessary that the measurement of NT should be standardized and subjected to ongoing quality assurance.

These methods problems are further highlighted by a study of 47,053 singleton pregnancies that were examined at 6 to 16 weeks of gestation.[137] In 11,025 of the patients (23.4%), no valid NT measurement was taken because the scans were carried out at inappropriate gestations (n=4228 pregnancies) or the sonographers were unable to obtain a measurement (n=3416 pregnancies) or none of the images were deemed to be of an acceptable quality (n=3881 pregnancies).[137]

Further evidence on the difference between observational and interventional studies is provided by Crossley et al.[136] In this observational study, 17,229 pregnancies were recruited, and fetal NT was measured successfully in 72.9% of cases. In a subsequent study of >2000 pregnancies in which the results of the scan were given to the women, fetal NT was measured successfully in 99.8% of cases.[136]

Fetal NT and maternal serum biochemistry

Trisomic pregnancies are associated with altered maternal serum concentrations of various fetoplacental products, which included α-fetoprotein (AFP), free β–human chorionic gonadotropin (β-hCG), inhibin A, and unconjugated estriol.[138-142] Screening by maternal age and various combinations of these fetoplacental products can identify 60% to 75% of trisomy 21 pregnancies, which represents a false-positive rate of 5%.[143] However, an essential component of biochemical screening is the accurate dating of the pregnancy by ultrasound, otherwise the detection rate is reduced by approximately 10%.

Table VII Prospective first-trimester screening studies by fetal NT and maternal serum free β-hCG and PAPP-A that provided data on the detection rate for trisomy 21 at a 5% false-positive rate

Study/year	Gestation (wk)	N	Detection rate (n/N)
Krantz et al/2000[150]	$10\text{-}13^{+6}$	5,809	30/33 (90.9%)
Bindra et al/2002[151]	$11\text{-}13^{+6}$	14,383	74/82 (90.2%)
Spencer et al/2000[152]; 2003[153]	$10\text{-}13^{+6}$	11,105	23/25 (92.0%)
Schuchter et al/2002[154]	$10\text{-}13^{+6}$	4,802	12/14 (85.7%)
Wapner et al/2003[155]	$10\text{-}13^{+6}$	8,514	48/61 (78.7%)
Total		44,613	187/215 (87.0%)

Fetal NT and maternal serum testing in the first-trimester

In trisomy 21 pregnancies at 10^{+3} to 13^{+6} weeks of gestation, the maternal serum concentration of free β-hCG is higher than in chromosomally normal fetuses, whereas pregnancy-associated plasma protein-A (PAPP-A) is lower (approximately 2 MoM and 0.5 MoM, respectively).[138,144-148]

There is no significant association between fetal NT and maternal serum free β-hCG or PAPP-A in either trisomy 21 or chromosomally normal pregnancies; therefore, the ultrasonographic and biochemical markers can be combined to provide more effective screening than either method individually.[146-149] In a retrospective study of 210 singleton pregnancies with trisomy 21 and 946 chromosomally normal control subjects who were matched for maternal age, gestation, and sample storage time, we estimated that the detection rate for trisomy 21 by a combination of maternal age, fetal NT, and maternal serum PAPP-A and free β-hCG would be approximately 90%, which represents a screen-positive rate of 5%.[149]

Six prospective screening studies have confirmed the feasibility and effectiveness of combining fetal NT and maternal serum free β-hCG and PAPP-A (Table VII).[150-155] The study of Bindra et al[151] also reported the detection rates for fixed false-positive rates between 1% and 5% (Table VIII) and the false-positive rates for fixed detection rates between 60% and 90% (Table IX) of screening for trisomy 21 by maternal age alone, maternal age and fetal NT, maternal age, and serum free β-hCG and PAPP-A and by maternal age, fetal NT, and maternal serum biochemistry. Thus, for a 5% false-positive rate, the detection rate of trisomy 21 by the first-trimester combined test was 90%, which is superior to the 30% that was achieved by maternal age and 65% that was achieved by second-trimester biochemistry. Alternatively, the detection rate of 65% that was achieved by second-trimester biochemical testing with a 5% false-positive rate can be achieved by first-trimester combined testing, with a false-positive rate of only 0.5%.[151]

In trisomies 18 and 13, maternal serum free β-hCG and PAPP-A are decreased.[156,157] In cases of sex chromosomal anomalies, maternal serum free β-hCG is normal, and PAPP-A is low.[158] In diandric triploidy, maternal serum free β-hCG is increased greatly, whereas PAPP-A is decreased mildly.[159] Digynic triploidy is associated with markedly decreased maternal serum free β-hCG and PAPP-A.[159] Screening by a combination of fetal NT and maternal serum PAPP-A and free β-hCG can identify approximately 90% of all these chromosomal abnormalities, which represents a screen positive rate of 1%.

An important development in biochemical analysis is the introduction of a new technique (random access immunoassay analyzer with time-resolved amplified cryptate emission), which provides automated, precise, and reproducible measurements within 30 minutes of obtaining a blood sample. This has made it possible to combine biochemical and ultrasonographic testing and to counsel in 1-stop clinics for early assessment of fetal risk.[151-153]

Fetal NT and maternal serum testing in the second trimester

In women who undergo second-trimester biochemical testing after first-trimester NT screening the a priori risk must be adjusted to take into account the first-trimester screening results.[9] Supportive evidence is provided by the findings of 3 prospective studies that examined the impact of first-trimester screening by NT on second-trimester serum biochemical testing.[129,160,161] In 1 study, the proportion of affected pregnancies in the screen-positive group (positive predictive value) with the second-trimester double maternal serum test was 1 in 40 pregnancies, but after the introduction of screening by NT, 83% of trisomy 21 pregnancies were identified in the first trimester, and the positive predictive value of biochemical testing decreased to 1 in 200 pregnancies.[160] In the second study, first-trimester screening by NT identified 71% of trisomy 21 pregnancies, which represents a screen-positive rate of 2%; the positive predictive value of the second-trimester quadruple maternal serum test was only 1 in 150 pregnancies.[161] In the third study, 2683 women had NT screening; in 74 women (2.8%), the estimated risk for trisomy 21 was 1 in ≥100 pregnancies; this group contained all 8 cases of trisomy 21.[129] In the 2609 screen-negative women, 1057 women agreed to have the second-trimester triple maternal serum test, and 1552 women (59.5%) declined further screening. In the 1057 women who had serum testing, 46 women (4.4%) had an estimated risk for trisomy 21 of 1 in ≥250 pregnancies, and these were all false positive.[129]

Table VIII Detection rates for different fixed false-positive rates in screening for trisomy 21 by the combination of maternal age, fetal NT, and maternal serum free β-hCG and PAPP-A*

	Fixed false-positive rate (n)				
Method of screening	1%	2%	3%	4%	5%
Maternal age	9 (11.0%)	14 (17.1%)	19 (23.2%)	23 (28.0%)	25 (30.5%)
β-hCG and PAPP-A	22 (26.8%)	33 (40.2%)	39 (47.6%)	42 (51.2%)	49 (59.8%)
NT	53 (64.6%)	60 (73.2%)	62 (75.6%)	64 (78.0%)	65 (79.3%)
NT and β-hCG and PAPP-A	63 (76.8%)	65 (79.3%)	69 (84.1%)	72 (87.8%)	74 (90.2%)

* In this population of 14,383 pregnancies, there were 82 cases of trisomy 21.[151]

Table IX False positive rates for different fixed detection rates in screening for trisomy 21 by the combination of maternal age, fetal NT and maternal serum free β-hCG and PAPP-A*

	Fixed sensitivity (n)						
Method of screening	60%	65%	70%	75%	80%	85%	90%
Maternal age	1993 (14.0%)	2724 (19.1%)	3577 (25.1%)	3939 (27.7%)	4782 (33.6%)	6603 (46.4%)	7537 (52.9%)
β-hCG and PAPP-A	723 (5.1%)	815 (5.7%)	1002 (7.0%)	1433 (10.1%)	1866 (13.1%)	2167 (15.2%)	2594 (18.2%)
NT	80 (0.6%)	140 (1.0%)	193 (1.4%)	367 (2.6%)	874 (6.1%)	1299 (9.1%)	2276 (16.0%)
NT and β-hCG and PAPP-A	37 (0.3%)	68 (0.5%)	91 (0.6%)	128 (0.9%)	305 (2.1%)	432 (3.0%)	718 (5.0%)

* In this population, there were 14,240 normal and 82 trisomy 21 pregnancies.[151]

Three studies reported on prospective screening by a combination of fetal NT in the first trimester and maternal serum biochemistry in the second trimester.[111,123,124] They classified as screen positive those women with increased fetal NT (above a cut off of 2.5 mm[111] or 3.0 mm[123,124]) and those women with a maternal serum screening–derived risk of 1 in $\geq$ 250 pregnancies. In 2 of the studies, most of the patients had both components of the test. For a combined screen positive rate of 6.5%, the detection rate of trisomy 21 was 93.5% (Table X).[111,123] If screening had been only by NT, the detection rate would have been 58.1%, which represents a screen positive rate of 2.3%. In the third study of 9118 patients, 5506 women had both components of the test; 821 women had only ultrasound testing, and 2791 women had only serum biochemistry.[124] For an invasive testing rate of 8.6%, 17 of the 21 fetuses (81.0%) with trisomy 21 were detected. In the subgroup of 6234 women who had NT screening, the fetal NT was $\geq$ 3 mm in 3% of cases, which included 13 of the 21 women (61.9%) with trisomy 21.

These results demonstrate that, in prospective interventional 2-stage studies for an invasive testing rate of approximately 2.5%, approximately 60% of trisomy 21 pregnancies can be detected in the first trimester because of increased fetal NT. Second-trimester serum testing will result in invasive testing in a further 4% to 5% of pregnancies to identify a further 30% of affected fetuses. The same detection rate with a lower false-positive rate can be achieved by combining fetal NT with biochemical testing in the first trimester (Table VIII).

Integration of first and second trimester testing

A statistical model that combined first-trimester fetal NT and maternal serum PAPP-A with second-trimester free β-hCG, estriol, and inhibin A estimated that, for a false-positive rate of 5%, the detection rate of trisomy 21 could be 94%.[162] Even if the estimates of this hypothetic test are found to be true in prospective studies, it is unlikely that the test will gain widespread clinical acceptance.[163] This test assumes complete compliance by the pregnant women in participating in a 2-stage process that is separated by 1 month, in having an ultrasound scan without receiving information as to whether the fetus looks normal, and in accepting second- rather than first-trimester diagnosis and termination.

Some of the logistical problems in the implementation of an integrated test are highlighted by the results of a multicenter observational serum, urine, and ultrasound screening study that investigated first and second trimester screening for trisomy 21.[136] The aim of the study was to obtain a measurement of fetal NT in the first trimester and to collect maternal serum and urine samples in the first and second trimesters. Intervention was based on the second-trimester serum results, and all other data were analyzed retrospectively. However, of the 47,053 women with singleton pregnancies who were recruited, 9691 women (20.6%) did not attend for second-trimester serum testing, and all components of the protocol were completed in only 28,434 of the women (60.4%).[136]

Table X Prospective screening by a combination of fetal NT thickness in the first trimester and maternal serum biochemistry in the second trimester

		Increased NT		Combined test	
Study/year	N	Screen positive (%)	Detection rate (n/N)	Screen positive (%)	Detection rate (n/N)
Schuchter et al/2001[111]	9,342	2.4	11/19 (57.9%)	7.2	18/19 (94.7%)
Audibert et al/2001[123]	4,130	2.0	7/12 (58.3%)	5.0	11/12 (91.7%)
Total	13,472	2.3	18/31 (58.1%)	6.5	29/31 (93.5%)

Table XI Differences in false-positive and detection rates in screening for trisomy 21 by fetal NT and serum biochemical testing between prospective studies and various theoretic modeling techniques

Study/year	Type of study	N	Screening test	False-positive rate (%)	Detection rate (%)
Wald et al/2003[137]	Retrospective statistical model	30,375	Maternal age & second-trimester double test	5.0	71
Wald et al/2003[164]	Prospective study	46,193	Maternal age & second-trimester double test	5.0	61
Wald et al/2003[137]	Retrospective statistical model	30,375	Maternal age & second-trimester triple test	5.0	77
Wald et al/2003[164]	Prospective study	46,193	Maternal age & second-trimester triple test	5.0	66
Wald et al/2003[137]	Retrospective statistical model	30,375	Maternal age & second-trimester quadruple test	5.0	83
Wald et al/2003[164]	Prospective study	46,193	Maternal age & second-trimester quadruple test	5.0	75
Wald et al/2003[137]	Retrospective statistical model	30,375	NT	5.0	34
Studies in Table III	Prospective studies	200,868	NT	4.2	77
Wald et al/2003[137]	Retrospective statistical model	30,375	Maternal age & first & second trimester integrated	5.0	93
Malone et al/2004[165]	Retrospective statistical model	33,557	Maternal age & first & second trimester integrated	5.4	90
Studies in Table VII[150-155]	Prospective studies	44,613	Maternal age & first-trimester NT, β-hCG, PAPP-A	5.0	87
Wald et al/1999[162]	Theoretical model	-	Maternal age & first & second trimester integrated	1.0	85
Wald et al/2003[137]	Retrospective statistical model	30,375	Maternal age & first & second trimester integrated	1.2	85
Malone et al/2004[164]	Retrospective statistical model	33,557	Maternal age & first & second trimester integrated	2.8	85
Bindra et al/2002[151]	Prospective study	14,383	Maternal age & first-trimester NT, β-hCG, PAPP-A	3.0	85

In this study, there were 101 fetuses with trisomy 21.[136] The data from the 75 cases with satisfactory NT images were then used to develop a model that was based on the combination of fetal NT and PAPP-A at 9 to 10 weeks of gestation and maternal serum free β-hCG, inhibin-A, unconjugated estriol, and AFP at 14 to 20 weeks of gestation. According to this statistical model, for a 5% false-positive rate, 93% of trisomy 21 fetuses could be detected.[136] However, it is likely that this model is inaccurate. For example, the predicted detection rates, for a 5% false-positive rate, were 71% for the double test, 77% for the triple test and 83% for the quadruple test, which are substantially higher than the respective rates of 61%, 66%, and 75% that were reported by the same authors in their prospective screening studies (Table XI).[164]

A similar study in the United States (FASTER trial) reported its findings in the subgroup of 33,557 pregnancies with complete first and second trimester data, which included 84 cases of trisomy 21.[165] It was estimated that, for a 5.4% false-positive rate, 90% of trisomy 21 fetuses could be detected. However, the prospective studies that

are summarized in Table VII have demonstrated that such results are achievable by screening with fetal NT and maternal serum free β-hCG and PAPA-A in the first trimester.

In the initial theoretic model of the integrated test, it was estimated that, for a detection rate of 85%, the false-positive rate would be 1%,[162] which was revised to 1.2% after retrospective statistical modeling of the serum, urine, and ultrasound screening study results[164] and further revised to 2.8% after modeling of the FASTER results (Table XI).[165] In the prospective study of Bindra et al,[151] the detection rate of 85% was achieved with a false-positive rate of 3.0% by screening with fetal NT and maternal serum free β-hCG and PAPA-A in the first trimester.

NT followed by second-trimester ultrasonography

In the first trimester, a common feature of many chromosomal abnormalities is increased NT thickness. In the second trimester scan, each chromosomal defect has its own syndromal pattern of detectable abnormalities. For example, trisomy 21 is associated with nasal hypoplasia, increased nuchal fold thickness, cardiac defects, intracardiac echogenic foci, duodenal atresia and echogenic bowel, mild hydronephrosis, shortening of the femur and more so of the humerus, sandal gap, and clinodactyly or mid-phalanx hypoplasia of the fifth finger.

In women with second-trimester sonographic markers of chromosomal abnormalities after first-trimester screening, the a priori risk must be adjusted to take into account the first-trimester screening results. On the basis of existing data, the likelihood ratio for trisomy 21, if there is no detectable defect or marker, is 0.30. The estimated positive and negative likelihood ratios are 53.0 and 0.67 for increased nuchal fold thickness, 22.8 and 0.68 for short humerus, 7.9 and 0.62 for short femur, 6.8 and 0.85 for mild hydronephrosis, 6.4 and 0.75 for intracardiac echogenic foci, and 21.2 and 0.87 for echogenic bowel.

Additional information and references are provided on the website of the American Journal of Obstetrics and Gynecology.

Absence of fetal nasal bone

In 1866 Down noted that a common characteristic of patients with trisomy 21 is a small nose.[1] An anthropometric study in 105 patients with Down syndrome at 7 months to 36 years of age reported that the nasal root depth was abnormally short in 49.5% of cases.[166] In the combined data from 4 postmortem radiologic studies in a total of 105 aborted fetuses with trisomy 21 at 12 to 25 weeks of gestation, there was absence of ossification of the nasal bone in 32.4% of cases and nasal hypoplasia in 21.4% of cases.[167-170] Sonographic studies at 15 to 24 weeks of gestation reported that approximately 65% of trisomy 21 fetuses have absent or short nasal bone.[171-175]

Sonographic assessment

The fetal nasal bone can be visualized by sonography at 11^{+0} to 13^{+6} weeks of gestation.[176] This examination requires that the image is magnified so that the head and the upper thorax only are included in the screen (Figures 1 and 2). A mid-sagittal view of the fetal profile is obtained with the ultrasound transducer being held parallel to the longitudinal axis of the nasal bone. The angle of insonation is crucial because the nasal bone will not be visible almost invariably when the longitudinal axis of the bone is perpendicular to the ultrasound transducer. In the correct view, there are 3 distinct lines. The first 2 lines, which are proximal to the forehead, are horizontal and parallel to each other, resembling an "equal sign." The top line represents the skin and the bottom line, which is thicker and more echogenic than the overlying skin, represents the nasal bone. A third line, which is almost in continuity with the skin but at a higher level, represents the tip of the nose. When the nasal bone line appears as a thin line, less echogenic than the overlying skin, it suggests that the nasal bone is not yet ossified and is classified therefore as being absent.

A study that investigated the necessary training of 15 sonographers with experience in measuring fetal NT to become competent in examining the fetal nasal bone at 11^{+0} to 13^{+6} weeks of gestation has demonstrated that the number of supervised scans that are required is on average 80, with a range of 40 to 120 scans.[177] Another study of 501 consecutively scanned fetuses by experienced sonographers reported that the fetal nasal bone can be examined successfully and measured in all cases without extension of the length of time that is required for scanning.[178]

Association with chromosomal abnormalities

Several studies have demonstrated a high association between absent nasal bone at 11^{+0} to 13^{+6} weeks of gestation and trisomy 21 and other chromosomal abnormalities (Tables XII and XIII).[176,179-186] In the combined data from these studies on a total of 15,822 fetuses, the fetal profile was examined successfully in 15,413 cases (97.4%), and the nasal bone was absent in 176 of 12,652 chromosomally normal fetuses (1.4%) and in 274 of 397 fetuses (69.0%) with trisomy 21. An important finding of these studies was that the incidence

Table XII Studies that reported about the incidence of absent nasal bone in first-trimester trisomy 21 fetuses

Study/year	Type of study	Gestation (wk)	N	Successful examination (%)	False-positive rate (%)	Detection rate of trisomy 21 (n/N)
Cicero et al/2001[172]*	Before chorionic villi sampling	$11\text{-}13^{+6}$	701	100	0.5	43/59 (72.9%)
Otano et al/2002[179]	Before chorionic villi sampling	$11\text{-}13^{+6}$	194	94.3	0.6	3/5 (60.0%)
Zoppi et al/2003[180]	Screening	$11\text{-}13^{+6}$	5,532	99.8	0.2	19/27 (70.0%)
Orlandi et al/2003[181]	Screening	$11\text{-}13^{+6}$	1,089	94.3	1.0	10/15 (66.7%)
Viora et al/2003[182]	Screening	$11\text{-}13^{+6}$	1,906	91.9	1.4	8/10 (80.0%)
Senat et al/2003[183]	Retrospective	$11\text{-}13^{+6}$	1,040	91.9	0.4	3/4 (75.0%)
Wong et al/2003[184]	Before chorionic villi sampling	$11\text{-}13^{+6}$	143	83.2	0.9	2/3 (66.7%)
Cicero etal/2003[185]*	Before chorionic villi sampling	$11\text{-}13^{+6}$	3,829	98.9	2.8	162/242 (67.0%)
Cicero etal/2004[186]	Before chorionic villi sampling	$11\text{-}13^{+6}$	5,918	98.9	2.5	229/333 (68.8%)
TOTAL			15,822	97.4	1.4	274/397 (69.0%)

* Included in Cicero et al 2004.[186]

Table XIII Incidence of absent nasal bone at 11 to 13^{+6} weeks of gestation in chromosomally abnormal fetuses[186]

Chromosomal abnormality	Absent nasal bone (n/N)
Trisomy 21	229/333 (68.8%)
Trisomy 18	68/124 (54.8%)
Trisomy 13	13/38 (34.2%)
Triploidy	0/19 (0)
Turner's syndrome	5/46 (10.9%)
XXY, XXX, XYY	1/20 (5.0%)
Other	8/48 (16.7%)

of absent nasal bone decreased with fetal crown-rump length, increased with NT thickness, and was substantially higher in Afro-Caribbean pregnancies than in white pregnancies. Consequently, in the calculation of likelihood ratios in screening for trisomy 21, adjustments must be made for these confounding factors.[185,186]

In contrast with the aforementioned studies, Malone et al[187] reported that they were able to examine the fetal nose in only 75.9% of 6316 fetuses who were scanned at 10 to 13 weeks of gestation and that the nasal bone apparently was present in all 9 of their trisomy 21 fetuses. However, the image that they published to illustrate their technique reports the nasal bone at the tip rather than the base of the nose.[188] Similarly, De Biasio and Venturini,[189] who retrospectively examined the photographs that were obtained for the measurement of fetal NT reported that the nasal bone was present in all 5 fetuses with trisomy 21. However, all 5 images that they published were inappropriate, both for the measurement of fetal NT and for the examination of the nasal bone, because they were either too small or the fetus was too vertical or too oblique.

The conclusion can be drawn that, at 11^{+0} to 13^{+6} weeks of gestation, the fetal profile can be examined successfully in >95% of cases and that the nasal bone is absent in approximately 70% of trisomy 21 fetuses and in approximately 55% of trisomy 13 fetuses. In chromosomally normal fetuses, the incidence of absent nasal bone is <1% in white populations and approximately 10% in Afro-Caribbean populations. Consequently, the absence of the nasal bone is an important marker of trisomy 21. However, it is imperative that sonographers who undertake risk assessment by examination of the fetal profile receive appropriate training and certification of their competence in performing such a scan.

Integrated sonographic and biochemical screening in the first trimester

A case-control study comprised of 100 trisomy 21 and 400 chromosomally normal singleton pregnancies at 11^{+0} to 13^{+6} weeks of gestation examined the potential performance of screening for trisomy 21 by a combination of sonography for the measurement of fetal NT and the assessment of the presence or absence of the fetal nasal bone and measurement of maternal serum free β-hCG and PAPP-A at 11^{+0} to 13^{+6} weeks of gestation. It was estimated that, for a false-positive rate of 5%, the detection rate of trisomy 21 would be 97% and, for a false-positive rate of 0.5%, the detection rate would be 91% (Table XIV).[190]

Other sonographic markers in the first trimester

In addition to increased NT, chromosomal abnormalities are associated with a pattern of characteristic

sonographic findings in the first trimester. Trisomy 21 is associated with abnormal flow velocity patterns in the ductus venosus and maxillary hypoplasia. In trisomy 18, there is early onset fetal growth restriction, bradycardia in approximately 20% of cases, exomphalos in 30% of cases, absent nasal bone in 55% of cases, and single umbilical artery in 75% of cases. Trisomy 13 is characterized by fetal tachycardia, which is observed in approximately two thirds of the cases, early onset fetal growth restriction, and megacystis, holoprosencephaly, or exomphalos in approximately 40% of the cases.[191] Turner syndrome is characterized by fetal tachycardia, which is observed in approximately 50% of the cases, and early onset fetal growth restriction.[192] In triploidy, there is early onset asymmetric fetal growth restriction, bradycardia in 30% of cases, holoprosencephaly, exomphalos or posterior fossa cyst in approximately 40% of cases, and molar changes in the placenta in approximately one third of cases.

The website of the American Journal of Obstetrics and Gynecology provides additional information and references on crown-rump length, fetal heart rate, maxillary length, ear length, femur and humerus length, single umbilical artery, megacystis, exomphalos, choroid plexus cysts, pyelectasis and cardiac echogenic foci, placental volume, and Doppler findings in the ductus venosus, uterine arteries, umbilical arteries and vein, jugular vein, and carotid artery.

Chromosomal abnormalities in multiple pregnancies

In multiple pregnancies that are compared with singleton pregnancies, prenatal diagnosis of chromosomal abnormalities is complicated because the techniques of invasive testing may provide uncertain results or may be associated with higher risks of miscarriage and because the fetuses may be discordant for an abnormality, in which case 1 of the options for the subsequent management of the pregnancy is selective feticide.

Selective feticide can result in spontaneous abortion or severe preterm delivery, which may occur several months after the procedure. The risk for these complications is related to the gestation at feticide. Selective feticide after 16 weeks of gestation is associated with a 3-fold increase in risk compared with reduction before 16 weeks of gestation, and there is an inverse correlation between the gestation at feticide with the gestation at delivery.[193]

Amniocentesis in twins is effective in providing a reliable karyotype for both fetuses; the procedure-related fetal loss rate is approximately 2%.[194] In the case of chorionic villus sampling, the procedure-related fetal loss rate is approximately 1%, but in approximately 1% of cases, there may be a diagnostic error, either because of sampling the same placenta twice or cross-contamination.[195-197] The main advantage of chorionic villus sampling is that it provides results sufficiently early to allow for safer selective feticide.

Table XIV Integrated first-trimester sonographic and biochemical screening for trisomy 21*

	Detection rate (%)	
False positive rate (%)	NT, free β-hCG, and PAPP-A	NT, nasal bone, free β-hCG, and PAPP-A
0.5	70	91
1.0	75	94
2.0	80	95
3.0	84	96
4.0	86	97
5.0	89	97

*Estimated detection rates for different fixed false-positive rates with the use of various marker combinations with maternal age.[190]

Screening by maternal age

In dizygotic pregnancies, the maternal age-related risk for chromosomal abnormalities for each twin may be the same as in singleton pregnancies; therefore, the chance that at least 1 fetus is affected by a chromosomal defect is twice as high as in singleton pregnancies. Furthermore, because the rate of dizygotic twinning increases with maternal age, the proportion of twin pregnancies with chromosomal abnormalities is higher than in singleton pregnancies. In monozygotic twins, the risk for chromosomal abnormalities is the same as in singleton pregnancies, and in most cases both fetuses are affected. The relative proportion of spontaneous dizygotic to monozygotic twins in white populations is approximately 2-to-1; therefore, the prevalence of chromosomal abnormalities that affect at least 1 fetus in twin pregnancies would be expected to be approximately 1.6 times that in singleton pregnancies.

Chorionicity can be determined reliably by ultrasonography in early pregnancy.[198,199] In counseling parents, it is possible to give more specific estimates of 1 and/or both fetuses who are being affected, depending on chorionicity. Thus, in monochorionic twins, the parents can be counseled that both fetuses would be affected and that this risk is similar to that in singleton pregnancies. If the pregnancy is dichorionic, then the parents can be counseled that the risk of discordancy for a chromosomal abnormality is approximately twice that in singleton pregnancies, whereas the risk that both fetuses would be affected can be derived by squaring the singleton risk ratio. This is in reality an oversimplification, because unlike monochorionic pregnancies that

are always monozygotic, only approximately 90% of dichorionic pregnancies are dizygotic.

Screening by second-trimester maternal serum biochemistry

In singleton pregnancies, screening for trisomy 21 by a combination of maternal age and second-trimester maternal serum biochemistry can detect 50% to 70% of trisomy 21 cases, which represents a 5% false-positive rate.[159] In twin pregnancies, the median value for maternal serum markers (such as AFP, hCG, free β-hCG, and inhibin-A) are approximately twice those for singleton pregnancies.[200] When this is taken into account in the mathematic modeling for calculation of risks, the estimate was that serum screening in twins may identify approximately 45% of affected fetuses, which represents a 5% false positive rate.

Screening by fetal NT thickness

Pandya et al[201] reported that, in 9 twin pregnancies that were discordant for trisomies 21 or 18, fetal NT at 10^{+3} to 13^{+6} weeks of gestation was >2.5 mm in 8 of the 9 trisomic fetuses and in 1 of the 9 chromosomally normal fetuses. Maymon et al[202] examined 174 twin pregnancies; the fetal NT was >95th percentile in 16 of the fetuses (4.6%), which included all 5 chromosomally abnormal fetuses. A study of 60 twin pregnancies, in which fetal NT was measured in the first trimester and maternal serum biochemistry testing was performed in the second trimester, reported that the false-positive rates of the 2 screening methods were 5% and 15%, respectively. There was 1 fetus with trisomy 21 that was identified by NT screening.[203]

In a screening study for trisomy 21 in 448 twin pregnancies, NT thickness was >95th percentile of the normal range, for crown-rump length in singleton pregnancies, in 7 of the 8 pregnancies (87.5%) with trisomy 21.[204] In the chromosomally normal group, the prevalence of increased NT was higher in fetuses from monochorionic pregnancies (8.4%) than in fetuses from dichorionic pregnancies (5.4%). The prevalence of pregnancies with increased NT in at least 1 of the fetuses in 13.7% of the monochorionic pregnancies and in 11.1% of the dichorionic pregnancies. Another study reported that the incidence of increased fetal NT in at least 1 fetus is higher in monochorionic (7/30 pregnancies; 23.3%) than in dichorionic (10/70 pregnancies; 14.3%) twin pregnancies.[205] In a series of 303 monochorionic pregnancies, fetal NT was >95th percentile in 52 of the 606 fetuses (8.6%) and in at least 1 in 41 fetuses (13.5%) of the 303 pregnancies. There were 2 cases of both fetuses being affected by trisomy 21; in 1 case, the NT was increased in both fetuses (3.1 mm and 2.4 mm at 11 weeks of gestation), but in the second case, the NT was increased only in 1 of the fetuses (8.2 mm and 1.8 mm at 13 weeks of gestation).[206]

These findings suggest that, in dichorionic twin pregnancies, the detection rate and false-positive rate of fetal NT in screening for trisomy 21 are similar to those in singleton pregnancies. Therefore, effective screening and diagnosis of major chromosomal abnormalities can be achieved in the first trimester, which allows the possibility of earlier and safer selective feticide for those parents who choose this option. An important advantage of screening by fetal NT in dichorionic twins is that, when there is discordancy for a chromosomal abnormality, the presence of a sonographically detectable marker helps to ensure the correct identification of the abnormal twin, should the parents choose selective termination.

In monochorionic pregnancies, the false-positive rate of NT screening is higher than in singleton pregnancies, because increased NT is an early manifestation of twin-to-twin transfusion syndrome.[206,207] The number of cases that were examined is still too small to draw definite conclusions as to whether, in the calculation of risk of trisomy 21 in monochorionic pregnancies, the NT of the fetus with the largest or the smallest measurement (or the average of the 2 measurements) should be considered.

Screening by fetal NT thickness and maternal serum biochemistry

In a prospective screening study by fetal NT, maternal serum free β-hCG was measured in 4181 singleton and 148 twin pregnancies. In the twin pregnancies, there were 12 pregnancies with trisomy 21. In the normal twin pregnancies, compared with singleton pregnancies, the median maternal serum free β-hCG adjusted for maternal weight was 1.94 MoM. In the 12 trisomy 21 twin pregnancies, the median level of free β-hCG was significantly higher than in normal twin pregnancies.[208] In a study of 159 twin pregnancies, the average free β-hCG was 2.1 times greater and the PAPP-A was 1.9 times greater than in 3466 singleton pregnancies. With statistical modeling techniques, the prediction was that, at a 5% false-positive rate, screening by a combination of fetal NT and maternal serum biochemistry would identify approximately 80% of trisomy 21 pregnancies.[209] In a prospective screening study in 206 twin pregnancies, the false-positive rate was 9.0% of pregnancies (19/206) and 6.9% of fetuses (28/412), and the detection rate of trisomy 21 was 75% (3/4 pregnancies).[210]

Screening in higher-order multiple pregnancies

Fetal NT is the only reliable method of screening for chromosomal abnormality in multifetal pregnancies. In

a study of 79 fetuses from 24 pregnancies with ≥3 fetuses that were conceived by assisted reproduction, fetal NT was measured successfully in all cases, and the distribution of measurements was similar to that in singleton pregnancies.[211]

Women's attitudes to first- versus second-trimester screening

Two studies have investigated the preference of pregnant women in terms of the methods of screening. In the first study, 43 of 224 women (19.2%) did not want to have any screening. In those who wanted screening, 177 of 181 of the women (97.8%) preferred the screening to be carried out in the first rather than in the second trimester.[212]

In the second study, 100 women who indicated an interest in having prenatal screening for Down syndrome were interviewed at their first hospital antenatal visit to assess their attitudes to first- versus second-trimester screening.[213] Women were told that the detection rates of the 2 methods were identical, and 74% of the women chose first-trimester NT screening rather than second-trimester serum screening. A criticism of NT screening has been that some women with increased fetal NT will face unnecessary decisions regarding invasive testing and ultimately pregnancy termination in an affected pregnancy that would otherwise have ended in spontaneous miscarriage.[214] In the survey of women's preferences, 69% of women stated that they would still choose NT screening, even if all the Down syndrome pregnancies that were identified by this method miscarried before the second trimester.[213] The women wanted to know whether their fetus had Down syndrome, regardless of the pregnancy outcome; they also valued the knowledge of an underlying reason for a miscarriage if it occurred.

Clinical importance of respect for autonomy

Respect for autonomy is a central principle in medical ethics and law.[215] This ethical principle obliges the physician to elicit and implement the patient's preferences. The relevance of respect for autonomy to first-trimester screening is 2-fold. First, early diagnosis of fetal abnormality and the option of early termination of pregnancy are important to many women. Second, most first-trimester screening tests provide reassurance for many women who would prefer not to have an invasive procedure if the risk is low.[216,217] Consequently, the provision of a high-quality first-trimester screening service significantly enhances the autonomy of pregnant women.[218]

Comment

Diagnosis of fetal chromosomal abnormalities requires invasive testing. Randomized studies have demonstrated that the risk of miscarriage from chorionic villus sampling in the first trimester is the same as for amniocentesis in the second trimester, provided these procedures are carried out by appropriately trained and experienced operators.

Most pregnant women prefer screening to be performed in the first rather than in the second trimester. The provision of a high-quality first-trimester screening service significantly enhances the autonomy of pregnant women.

The preference of women for first-trimester screening remains, even if they are told that all affected fetuses that are identified by first trimester screening miscarry before the second trimester. In reality, the rate of fetal death in trisomy 21 between 12 weeks of gestation and 16 weeks of gestation is <10%. There is evidence that increased NT does not necessarily identify those trisomic fetuses that are destined to die in utero and that with first-trimester screening the observed detection rate of trisomy 21 is only 2% to 3% higher than the potential rate of reducing the live birth incidence of this abnormality.

Prospective studies in >200,000 pregnancies, including >900 fetuses with trisomy 21, have demonstrated that NT screening can identify >75% of fetuses with trisomy 21 and other major chromosomal abnormalities, which represents a false-positive rate of 5%. This is superior to the 30% detection rate that is achieved by maternal age and 65% detection rate that is achieved by second trimester maternal serum biochemistry.

The recently introduced integrated test, which is claimed to be an effective method of screening, is a hypothetic test that is based on various statistical modeling techniques. It is unlikely that this test will gain widespread clinical acceptance, and it is likely that the real detection rate would be considerably lower and that the false-positive rate would be substantially higher than the original estimates.

Prospective studies, in >40,000 pregnancies, including >200 fetuses with trisomy 21, have demonstrated that first-trimester screening by a combination of fetal NT and maternal serum free β-hCG and PAPP-A can identify 85% to 90% of fetuses with trisomy 21, which represents a false-positive rate of 5%. This method can also identify >90% of fetuses with trisomies 18 and 13, Turner syndrome, and triploidy, which represents a screen-positive rate of 1%.

In dichorionic twin pregnancies, the measurement of NT in each fetus provides effective screening that leads to the diagnosis of major chromosomal abnormalities in the first trimester. This allows the possibility of earlier and safer selective feticide for those parents who choose

this option. In monochorionic pregnancies, the false-positive rate of NT screening is higher than in singleton pregnancies, because increased NT is an early manifestation of twin-to-twin transfusion syndrome and a marker of chromosomal abnormalities.

As with all aspects of good clinical practice, those operators who perform first-trimester scans should be trained appropriately, and their results should be subjected to external quality assurance. This process has been well established by the FMF several years ago and is accepted widely internationally.

Supplemental material for this article is available online at www.us.elsevierhealth.com/ajog

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